A TOXICITY STUDY ON

"GANDHAGA MATHIRAI"

Dissertation Submitted To

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY

Chennai – 32

For the Partial fulfillment in Awarding the Degree of

DOCTOR OF MEDICINE (SIDDHA)

(Branch – VI, Nanju Noolum Maruthuva Neethi Noolum)

Department of Nanju Noolum Maruthuva Neethi Noolum

Government Siddha Medical College

Palayamkottai – 627 002

OCTOBER – 2019

GOVT. SIDDHA MEDICAL COLLEGE, PALAYAMKOTTAI

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "**A Toxicity Study on GANDHAGA MATHIRAI**" is a bonafide and genuine research work carried out by me under the guidance of **Dr. M. THIRUTHANI**, **M.D(s).,** Professor & Head of the Department, Post Graduate Department of Nanju Noolum Maruthuva Neethi Noolum, Govt.Siddha Medical College, Palayamkottai, and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date: **Signature of the Candidate**

Place: Palayamkottai **Dr. S. INDUMATHI**

CERTIFICATE

This is to certify that the dissertation entitled **"A TOXICITY STUDY ON GANDHAGA MATHIRAI"** is a bonafide work done by **Dr. S. INDUMATHI (Reg.No. 321616001)** Govt. Siddha Medical College, Palayamkotai in partial fulfillment of the university rules and regulations for award for **MD(s) Nanju Noolum Maruthuva Neethi Noolum** under my guidance and supervision during the academic year 2016-2019.

Name and signature of the Guide :

Name and signature of the Head of Department:

Name and signature of the Principal:

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The Tamil Nadu Dr. M.G.R. Medical University

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This certificate is awarded to Dr/~~Mr/Mrs~~....S. INDUMATHI.....

for participating as ~~Resource Person~~ / Delegate in the XXIII Workshop on

"RESEARCH METHODOLOGY & BIOSTATISTICS"

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This is to certify that the dissertation topic **A Toxicity study on"GANDHAGA MATHIRAI"**has been approved by the screening committee.

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CERTIFICATE OF MINERAL AUTHENTICITY

Certified the following the Mineral (Thathu) drug used in Siddha formulation (Internal) **"KANTHAGA MATHIRAI"** for **"SEVVATTAIKADI, KAANAKADI, SEMPOORANKADI"** taken up for Post-Graduation Dissertation Studies by **Dr.S.INDUMATHI,** PG Scholar of MD Siddha, Department of Toxicology, have selected the raw drug(Minerals) and have been authenticated through Geological methods(Macro/Micro).

S.NO	DRUG	ENGLISH NAME	CHEMICAL NAME
1	Kanthagam	Sulphur	Sulphur

Station : Palayamkottai

Date: 24.12.2018

24/12/18

Authorized Signature

DR. G. ESSAKKYPANDIAN
Lecturer,
அரசினர் சித்த மருத்துவ கல்லூரி
மருத்துவமனை
பாளையங்கோட்டை

GOVERNMENT SIDDHA MEDICAL COLLEGE, PALAYAMKOTTAI

CERTIFICATE OF BOTANICAL AUTHENTICITY

Certified the following plants drugs used in Siddha formulation **"GANDHAGA MATHIRAI"** taken up for Post-Graduation Dissertation studies by **Dr. S. INDUMATHI,** PG Scholar MD Siddha, Department of Nanju Noolum Maruthuva Neethi Noolum, are correctly identified and authenticated through visual inspection / organoleptic characters / Experience and Training, Morphology, Microscopical and Taxonomical methods.

S. No.	Tamil Name (herbals)	Botanical Name	Family	Parts used
1	Changan Leaf	Azima tetracantha	Salvadoraceae	Leaf

Station: Palayamkottai

Date: 20/2/19.

20/2/19

Authorized Signature

Dr. S. SUTHA, M.Sc.,M.Ed.,Ph.D.,
Associate Professor
Dept. of Medicinal Botany
Govt. Siddha Medical College
Palayamkottai, Tirunelveli - 2.

NANDHACOLLEGE OF PHARMACY, ERODE - 52

Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA)
Institutional Animal Ethics Committee (IAEC)
Reg No: 688 /PO/Re/S/02/CPCSEA

CERTIFICATE

Title of the project	:	A TOXICITY STUDY ON GANDHAGA MATHIRAI
Proposal Number	:	NCP/IAEC/2018-19/20
Date received after modification (if any)	:	---
Date received after second modification	:	---
Approval date	:	27.12.2018
Species & Number of animals sanctioned	:	WISTER ALBINO RAT/42
Expiry date (Termination of the Project)	:	05.04.2019
Name of the IAEC / CPCSEA Nominee	:	Dr. C. Gunasekaran

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Scientist From Different Discipline

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S.No: 092

CERTIFICATE

This Certifies that

Dr. S. Indumathi

has participated in Continuing Medical Education on "**AYUSH External Therapies-II**" held at GSMCH, Palayamkottai on Dec, 4 2018

Dr. A.S.Poongodi Kanthimathi MD (s).,
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LIST OF TABLES

ABBREVIATIONS

GM	GANDHAGA MATHIRAI
No.	Number
Mg	Milligram
Kg	Kilogram
LD_{50}	Lethal $Dose_{50}$
ED_{50}	Effective $Dose_{50}$
p.o	peros
ML	Milliliter
%	percentage
R&D	Research and Development
EDTA	Ethylene Diamine Tetra Acetic Acid
M	Male
g%	Gram percentage
g	Gram
NOAEL	No-Observed-Adverse-Effect-Level
MLD	Minimum Lethal Dose
MTD	Maximum Tolerated Dose
OECD	Organization of Economic Co-operation and Development
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
FTIR	Fourier Transform – Infra Red Spectroscopy
SEM	Scanning Electron Microscopy
ICP-OES	Inductively Coupled Plasma Optical Emission-Spectrometry
LD	Low Dose
MD	Middle Dose
HD	High Dose
BDL	Below Detection Limit

1. INTRODUCTION

Siddha system of medicine is the oldest traditional system of medicine in world originated in state of Tamilnadu, India.

"Siddha is the divine gift of nature to mankind"

The siddha system of medicine is one of the ancient and native system medicine. Siddha system is handled by a group of religious. Personalities identified as siddhars. Siddha system of medicine was primarily sponsored and developed by siddhars in Tamil land . They were highly cultured, intellectual and spiritual faculties combined with divine aspects. Siddhars age is beyond our assessment. They considered that nature in man and man is nature. Man is nothing but a world in miniature containing the five elements and the various principles which constitue the mineral, the vegetable and the animal kingdoms. Man is the highest of all living beings.

Sattamuni siddhar says as follows,

அண்டத்தில் உள்ளதே பிண்டம்
பிண்டத்தில உள்ளதே அண்டம்
அண்டமும் பிண்டமும் ஒன்றெ
அறிந்துதான் பார்க்கும் போதே

\- சட்டமுனி ஞானம்

So the changes that occur in the universe will affect the physical body also hence the body will get upset or alter from normal, if there is any adverse change in the universe. Since both of them are formed by the same elements in different proportions.

In the siddha system of medicine the treatment for imbalance of the mukkutram are made up of the five elements (panchaboothas) The drugs are made up of five elements. Panchaboothams and mukkutram are all present in the "96" Thathuvas which are the basic of siddha system of medicine.

Tridoshas are involved in all functions of the body, physical, emotional and mental. They may be compared to be three pillars that support structure. The bodily activities, voluntary and involuntary are linked to vatham.

Pitham is linked to bodily changes involving destruction / metabolism. All constructive processes are performed by kapam. Their function dependent on each other.

Siddha system of medicine is concerned with herbals, minerals and their combinations. The early beginning of an art of healing with metals as knowledge of metals are found in magical stropes of "Atharvanaveda" and "Gowshika sutra". The use of metals and metalic in the field of medicine for combating major and minor ailments is as old as mankind.

These medicines can be administered in small doses. They are available in all seasons and can be preserved. The siddhars were aware of the metalic compounds and their knowledge was so advanced that they could prepare them from simpler materials. **Agasthiyar, Thirumoolar and Boagar** are three among the lineage of the 18 siddhars. They have contributed to the preparation of these medicines. As the universe is said of the panchaboothas, so as the medicines. Some of the methods by the siddhars still survive under a veil of secrecy, certain mercury and arsenic compounds are manufactured only in families and the methods are guarded secrecy.

Sulphur occupies a very high place in siddha medicine. It is used as catalytic agent in many of its medicines. When sulphur is used combination with mercury. The addition of sulphur is to control the fluidity of mercury.

Research is necessary to solve such apparent riddles of the transformation of these admitted poisonous compounds.

The above said medicines have a characteristic feature of treating a number of diseases as in short term and long term administration. By keeping all these facts in mind, I have selected "Gandhaga Mathirai" for my dissertations study.

2. AIM AND OBJECTIVES

AIM:

The main aim of this study is to access the safety of the drug "GANDHAGA MATHIRAI" on wistar albino rats under various dose levels of drug administration especially in acute and sub acute toxicity studies.

OBJECTIVE:

- To collect the literature and other evidences of each ingredient on pharmacological and toxicological aspect.
- To collect and purify the raw drugs according to literature evidence.
- To prepare the medicine based on the procedure quoted in literature.
- To establish the acute and sub acute toxicity of the drug.
- To evaluate the biochemical analysis of the drug.
- To analysis the haematological investigations and histopathological study of the organs such as kidney, liver, heart and brain in wister rats.
- To create an awareness among the practitioners of siddha to go for further study regarding the adverse effect in the drug.

3.REVIEW OF LITERATURE

கந்தகம்

SIDDHA ASPECT

வேறுபெயர்கள்

காரிழையின் நாதம், பரைவீரியம், அதீதப் பிரகாசம், பீஜம், செல்விவிந்து, சக்தி, சக்திபீசம், செந்தூரத்தாதி, தனம், தேவியுரம், நாதம், நாற்றம், பரைநாதம், பொன்வர்ணி, இரச சுரோணிதம்.

காணப்படும் இடங்கள்

தூய்மையாக இயல்பாகத் தோன்றிய இந்தப் பொருளானது எரிமலைச் சாரல்களிலும் பூமியில் அனேக இடங்களிலும் காணப்படும். கடைச்சரக்கானது முக்கியமாய், சிசிலி, இத்தாலி முதலிய நாடுகளிலிருந்து கொண்டு வரப்படுகின்றன.

கடையில் விற்கப்படும் கந்தகத்தில் அயம், நாகம், பாடாணம், சுண்ணாம்பு முதுலிய குற்றங்கள் இருக்கின்றன. ஆகையால் இதை உருக்கியாவது வாலையிலிட்டு இறக்கியாவது, பதப்படுத்தியாவது தூய்மை செய்து கொள்ள வேண்டும்.

- நேபாளம், காஷ்மீர், ஆப்கானிஸ்தானம், பர்மா
- தாது தாவர ஜீவப் பொருள்களிலும் களப்புற்றிருக்கின்றது.

வகைகள்

- வெண்மை நிறம் - எல்லா நோய்களையும் தீர்க்கும்
- கிளிமூக்குச் சிவப்பு நிறம் - நவலோகத்தை ஏமமாக்கும்.
- பொன்னை நிறம் - குற்றமற்ற நெல்லிக்காய் போன்று இருக்கும்.
- சூதகத்தோடு உறவாகிச் சுத்தமாய் இருக்கும்.
- காகத்தின் நிறம் - அகப்படாது, அகப்பட்டால் நரைதிரைகள் அற்றுப்போம்.
- பதார்த்த குண சிந்தாமணி கூறியிருப்பது

நெல்லிக்காய் கந்தகம், வாணகந்தகம் - மருந்துகளில் கையாளப்படுவது ஆகும்.

- கோழித்தலை கந்தகம்
- அமரசிலைக்கந்தகம்
- காட்டுக்கந்தகம்
- நெல்லிக்காய் கந்தகம்
- வாண (அ) குழாய்க் கந்தகம் எனப் பலவகைப்படும்.

ஆனால், சித்த, யுனானி இரசவாத மருத்துவர்கள்,

- சிவப்புக் கந்தகம்
- பசுஞ்சாயலான மீதகந்தகம்
- வெள்ளை கந்தகம்
- ஊதாக் கந்தகம் என 4 வகைகளாகச் சொல்கிறார்கள்.

சிவப்புக் கந்தகமானது – பிரகாசமுள்ளதாகவும், தன் சுரங்கத்தில் இராக் காலங்களில் தீபத்தைப் போன்ற ஒளியை உடையதாய் தனக்கடுத்திருக்கும் சுரங்கங்களிலும் வெளிச்சத்தை உண்டாக்கும் தன்மையுடையதாம்.

முக்கியமாய்ப் பார்சி நாட்டில் அம்மான்தீவில் இருக்கின்றன. நவாப் முஅத முதுல் முலூக் ஸையத் உல்விகான் என்னும் மகாபண்டிதர் தன்னுடைய நூலில் சொல்கின்றார். அம்மான் தீவிலுள்ள சில பாகங்களில் சிவப்புக் கந்தகத்தைப் பூமியிலிருந்து வெட்டியெடுதத்தை நான் நேரே பார்த்தேன் என்று, ஆனால் இவரது தலைமாணாக்கர் சிவப்புக் கந்தகத்தை தான் பார்த்ததேயில்லையென்கிறார்.

ஜாலிநியுஸ் பண்டிதர் சொல்கிறதாவது, சிவப்பு, மஞ்சள், வெள்ளை, கறுப்பு என்ற 4 வகைகளென்பன நிச்சயமென்பதே.

பாடாணங்கள் 64-ல் இதுவும் ஒன்று

பிறப்புக்கந்தகம் - மலையில் பிறக்கும்.

வைப்புக்கந்தகம், கோழித்தலைக் கெந்திவைப்பு, வாணிகெந்தி வைப்பு – பிறப்புக் கந்தியினை முதன்மையாகக் கொண்டு மற்றை சரக்குகளின் உதவியால் செய்யப்படுகின்ற சரக்குகளாகும்.

கோழித்தலை கெந்தியின் நிறம் - கோழித்தலைச் சூட்டின் நிறம்

இதற்கு வாசனையும் சுவையுமில்லை. ஆனால், இதைத் தேய்த்தால் ஒரு வித கெட்ட நாற்றம் உண்டாகும். கைக்கு நறநற வென்றிருக்கும். இது நீரில் கதையாது. கற்பூரதைலம், கொழுப்பு, மண்ணெண்ணெய், சாராயம் முதலியவற்றில் கரையும்.

இது எளிதில் நெருப்பில் பற்றத்தக்கது.

300 டிகிரிக்குக் காற்றுப்பட இ.்.து எரிக்கப்பட்டால் பற்றி எரியும். அதன் சுடரானது மங்கலான நீலநிறமாயிருக்கும். அதிகமாய் எரிக்கப்பட்டால் ஊதா நிறமாய் எரியும்.

180 டிகிரிக்கு எரிக்கப்பட்டால் அது ஆவியெழும்ப ஆரம்பிக்கும்.

216 டிகிரிக்கு எரிக்கப்பட்டால் இளகும்.

226 முதல் 280 டிகிரிக்கு எரிக்கப்பட்டால் முற்றும் நீராவடிவாகி இலேசான மஞ்சள் நிறம் உடையதாகும்.

320 டிகிரியானால் உருக்கின பாண்டத்தைத் தலைகீழாகக் கவிழ்த்தாலும் அது சொட்டாத விதமாக நன்றாய் இறுகிச் சிவந்து போகும்.

நட்புசரக்கு - இரசம்

“கன்திக்கினமு மிசரந்தா னென்றாரரே”

பகைசரக்கு – தாம்பிரம்

“சொல்லுமே தாம்பிரத்தை கெந்தி கொல்லும்.

சுவை

கைப்பு, துவர்ப்பு

செய்கை

- பித்தநீரை அதிகப்படுத்தும்
- மலமிளக்கி, உடற்றேற்றி, வியர்வை பெருக்கி, கிருமிநாசினி.
- சிறிய அளவில் கந்தகத்தை உள்ளுக்கு அருந்த அ.்.து உடம்பில் சேர்ந்து வியர்வை, பால், சிறுநீர் இவற்றின் வாயிலாக வெளிப்படுவதைக் காணலாம்.
- தோல், அசுகங்களின் சளிச்சவ்விலுள்ள கோளங்களின் சுரப்பை அதிகப்படுத்தும்.
- விரேகியில் சிறப்பாகச் செயல்பட்டு சுரப்பை அதிகப்படுத்தும்.
- கந்தகத்தை அதிக அளவில் அருந்த பேதியை உண்டு பண்ணும்.

பொதுக்குணம்

"நெல்லிக்காய்க் கந்திக்கு நீள்பதினெண் குட்டம்மந்தம்
வல்லை கவிசைகுன்ம வாயு கண்ணோய் - பொல்லா
விடக்கடிவன் மேகநோய் வீறுசுரம் பேதி
திடக்கிரசு ணீகபம்போந் தேர்."

பதினெண்குட்டம், மந்தம், கல்லீரல், வீக்கம், பெருவயிறு வகைகளுள் ஒன்றாகிய கவிசை, குன்மவாயு, கண்ணோய்கள், கொடுமையைச் செய்கின்ற விடக்கடிகள், நாட்பட்ட மேகநோய்கள், வாதசுரம், பேதி, நாட்பட்ட கிரகணி, கபம் முதலியன நீங்கும்.

கந்தகம், தாய் மகவை வளர்ப்பது போல நோய்களின் வெப்பத்தை மாற்றி உடம்பைத் தேற்றுவிக்கும் என்பதை,

"மாதர் மகவை வளர்ப்பதுபோ லேயுடம்பை
யாதரவா கத்தேற்றி யாக்கையினால் - மீதாக
மேவி யிடர்நோயின் வெப்பத்தை மாற்றுதலாற்
றேவியுர மென்பதுடல் தேர்"

- தேரன் பொருட்பண்பு

சுத்தி முறைகள்

- புளியம்பழ வோட்டைப் பற்றியிருக்கும் கசிவை ஊறவைத்திறுத்த நீர், காடிநீர், புளித்தமோர், காளான் சாறு இவைகளைத் தனித்தனி ஆறுபலமாக (210gm) எடுத்துக் கலந்து, ஒரு சட்டியிலிட்டு அச்சட்டிக்குச் சீலையினால் வேடுகட்டி, அதன்மேல் 1 பலம் 935gm) கந்தகத்தை வைத்து மூல்மூடி, அடுப்பேற்றி தீபாக்கினியாய் இரண்டு சாமம் (6மணி) எரிக்க, மலினம் மேல் தங்கி கந்தகம் சுத்தியாகிக் கீழிறங்கும்.
- மருதோன்றிக் கல்கத்தைப் பசுவின் தயிரில் கலந்து, ஒரு சட்டியிலிட்டுச் சீலையால் வேடுகட்டி, மேல் கந்தகத்தை வைத்து மற்றொரு சட்டியால் மூடிச் சீலை செய்து, குழியில் புதைத்து, மேல்சட்டிமேல் 5 வறட்டி கொண்டு புடமிட,

கந்தகம் உருகிக் கீழிறங்கும். சேகரித்துக் கொள்ளவும். இவ்விதம் 7 முறை செய்யவும்.

- கந்தகத்தை ஒரு இரும்புக் கரண்டியிலிட்டுச் சிறிது பசுவெண்ணெய் இட்டு உருக்கிப் பசும்பாலில் சாய்க்கவும். இவ்விதம் 30 முறை செய்யக் கந்தகம் சுத்தியாம். ஒவ்வொரு முறையும் புதிய பாலையே உபயோகிக்க வேண்டும்.

பாலுக்குப் பதில், வாழைக்கட்டை நீரில் கெந்தியைப் 10 முறை உருக்கி உருக்கிச் சாய்த்தெடுக்கச் சுத்தியாம். இம்முறையால் கந்தகத்திலுள்ள எண்ணெய் நீங்குமென்றும் கூறுவர்.

அளவு

10 (650mg) முதல் 30 (1.9gm) உளுந்தெடை

1 (4.2gm) முதல் 3 (12.6 gm) வராகனெடை கொடுக்க மலம் கழியும்.

கந்தகத்தின் சிறப்பு இயல்புகள் (அனுபோக வைத்திய நவநீதம் பாகம்-6)

கந்தகம் தேயுவின் குணம் சிறப்பாயிருக்கிறது. இதனால் சொற்பச் சூட்டாலும் இது உருகிவிடும். இதன் பூர்வீகமானது சந்திர சம்பந்தமானது. இரசமானது சூரிய சம்பந்தமானது. இவ்விரண்டிற்கு சக்தி சிவமென்றும் வாத வைத்திய வழக்கில் சொல்லப்படுகிறது.

இவ்விரண்டின் உதவியால் எல்லா உலோகங்களும் உற்பத்தியாகின்றன. தங்கம், வெள்ளி. தாம்பிரம், வெள்வங்கம், கருவங்கம், நாகம், இரும்பு என்னும் உலோகங்கள் மேற்கண்ட இரசம், கந்தகம் ஆகிய இரண்டும் அதனதன் அளவு, அதனதன் பாகம் ஆகியவைகளுக்குதத் தகுந்தபடி விளைகின்றன.

இரசமானது அகம்புறம் என்ற இரண்டு வகை நோய்களிலும் பயன்படுவது போலவே கெந்தகமும் பயன்படுகிறது.

கெந்தகமானது இரசத்தை பார்க்கிலும், மேன்மை யானதெனச் சொல்லப்பட்டிருக்கிறது. எப்படியெனில் இரசத்தை தனியே பயன்படுத்தினால் வாய்பிடிக்கிற குணமிருக்கிறது. கெந்தகத்தை தனியாக உபயோகித்தாலே அத்தனை தீக்குணம் செய்யாமல் இருப்பதுடன் இரசம், வீரம், பூரம் என்னும் சரக்குகளை முறை பிசகாய் உபயோகித்து வாய்பிடித்திருப்பதையும் நீக்கி, அவைகளின் வேகத்தை அடக்கி ஆரோக்கியத்தையுண்டாக்கும் சக்தியுடையது.

இரசம், கெந்தகம் ஆகியவைகள் முறைப்படி பாகப்படுத்தப்பட்டு, யாரிடத்திலிருக்கின்றனவோ அவர்கள் தாம் எல்லா நோய்களையும் போக்கக்கூடும் என்பது நிச்சயம்.

இதனால் செய்யப்பட்ட பற்பமானது எண்வகை பெரும் நோய்களான குட்டம், குன்மம், பாண்டு, சயம், பெருநோய், குறைநோய் முதலியவைகளை தீர்க்கும்.
இன்னும் கருங்குட்டம், வெண்குட்டம், செங்குட்டம் முதரலியவைகளும் தீரும்.

பலவகை நஞ்சுகள் இதனால் தீருகின்றன. பச்சைநாபி, கருநாபி, பாடாணம், அபினி முதலிய நஞ்சுகளெல்லாம் முறிந்து தீர்ந்து போகின்றன. இன்னும் பைத்தியநோய், பிரமை நோய் முதலிய நோய்களும் தீருகின்றன.

கந்தகமானது இரசவாதம், வைத்தியம் ஆகிய இருவகையிலும் முதன்மையானதாயிருப்பது போலவே துப்பாக்கி மருந்து, பீரங்கி மருந்து, வாணமருந்து முதலிய விநோத விசித்திர வேடிக்கைகளிலும் தேவையானதாயிருக்கிறது.

கந்தகத்தின் குணம்

தமிழ்

பதினெட்டுவகைக்குட்டம, மந்தநோய், வல்லைக்கட்டி, கவுசைநோய், பெருவயிறு, வாயுநோய்கள், குந்தநோய்கள், பெருநஞ்சுகள், மேகநோய்கள், வாதசுரம், வாதக்கழிச்சல், கபகிராணி, இருமல், இரைப்பிருமல், பைத்தியம் முதலிய நோய்கள் போம்.

அமிர்த சாகரம்

வாத, பித்தம், சிலேத்துமம் ஆகிய 3 வகைத் தோடங்களையும் நீக்கும். இரத்தகாசம், பெருநோய்கள், மண்ணீரல் நோய் ஆகியவைகளைக் கண்டிக்கும். சொறி, சிரங்கு, புண்கள், விரணங்கள் முதலிய தோல்நோய்களைக் கண்டிக்கும். நச்சு நுண்புழுக்களை நாசமாக்கும். மலத்தையிளக்கும். இரத்த விருத்தியை உண்டாக்கி முதுமைத் தன்மையைப் போக்கும்.

யூனானி

பியதாபேதகாரி (அதாவது தன்னுடைய சத்தினால் ஆழ்ந்து கிடக்கும் பழமையான சத்துக்களைக் கொஞ்சம் கொஞ்சமாக வெளிப்படுத்துவதாம்.)

நோய்த்தன்மையை மாற்றச் செய்யும் குட்டம், மண்ணீரல் சம்பந்தமான நோய்கள், மஞ்சள்நோய் ஆகியவைகளைக் கண்டிக்கும்.

சூதக இரத்தத்தை வெளிப்படுத்தும்

பிரமேக நோய்களைக் கண்டிக்கும்.

ஆங்கிலம்

மலத்தை இளக்கும் தன்மையுடையதாகையால், எருவாய் நோயாளிகள், கருப்பவதிகள் ஆகியவர்களுக்கு மலபந்தத்தை நீக்க அதிகமாக உபயோகப்படுத்தப்படுகிறது.

தோல் நோய்களுக்கு அகத்திலும், புறத்திலும் பயன்படுத்தப்படுகிறது.

கந்தக பேதம்

கந்தக பேதம் 4 வகை என குறிப்பிடப்பட்டுள்ளது. கந்தகத்தை,

“நாடியே பிரம்ம சத்திரியனோடு

நல்ல வைத்திய சூத்திரனாம் நாலுசாதி – என வகைப்படுத்துகிறார்.

- போகர் 7000

சுவேத கந்தகம் - விரணங்களுக்கு

ரக்த கந்தகம் - சுவர்ணாதி தாது மாரணங்களில்

பீதவர்ண கந்தகம் - ரசாயனங்களில்

கிருணகந்தகம் - சகல கிரியைகளில்

- அனுபவ வைத்தய தேவ இரகசியம்

பஞ்சபூதக்கூறு

"பாரப்பா பாடாணச் சரக்கில் பூதம்
பாடுகிறேன் தொட்டியுடன் பவழப் புற்று
ஆரப்பா கார்முகிலுஞ் சிங்கியோடு
அப்பனே தீமுறுக லஞ்சு மண்ணாம்
வேரப்பா குதிரைப்பல் கௌரி சங்கம்
வெள்ளையொடு சாவற் பூத் தண்ணீராகும்
சீரப்பா தாளகமுந் தாரங் கந்தி
சிலை வீரமிவையைந்துஞ் தீய தாமே"

- போகர் காரசாரத்துறை

"நாடியே தேய்வு நலமான கெந்தகந்
தோடு மனோசிலை திருவரி தாரமுங்
கூடிய வீரங் கூர்செம்புத் தொட்டியும்
பாடிய சொல்லும் பதிவான தேய்விதே"

- மச்சமுனி நாயனார் 800

என்ற செய்யுளாலும் கந்தகம் பஞ்சபூதக் கூறுபாட்டில் தேயு பூதத்தைச் சார்ந்ததாகும்.

வாத நோய்களுக்கு உபயோகமாகும் கந்தகம் சேரும் மருந்துகள்

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36. வாதராட்சச ரசம் - பக்கம் 412

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- நம்நாட்டு வைத்தியம்

சங்கன் - Azima tetracantha

வேறுபெயர்கள்

சங்கஞ்செடி, நற்சங்கன், முட்சங்கன்

பயன்படும் உறுப்புகள்:

இலை, வேர், பால்

காணப்படும் இடங்கள்

இ.ந்.து தென்னாட்டின், கீழ்க்கரை ஓரங்களிலும், இலங்கையிலும் பயிராகும் ஒருவகை முட்செடி , இலைதடித்தும், இலையின் நுனியில் முள்ளுள்ளதாயுமிருக்கும்.

சுவை : கைப்பு

தன்மை : வெப்பம்

பிரிவு : கார்ப்பு

செய்கை

- சிறுநீர்ப்பெருக்கி (Diuretic)
- வெப்பமுண்டாக்கி (Stimulant)
- துவர்ப்பி (Astringent)
- உரமாக்கி (Tonic)
- முறைவெப்பகற்றி (Antiperiodic)
- கோழையகற்றி (Expectorant)

குணம்

இதன் இலைக்கும் வேருக்கும் சோபை, கரப்பான், வெப்பம், கழலை, குன்மம், கீல்வீக்கம், வாதகோபம், பித்தநோய், பல நஞ்சுகள் இவை நீங்கும் - கண் துலக்கமும், மிகுதியும், குருதிப் பெருக்கும் உண்டாகும்.

“வீக்கம் கரப்பான் விதாகம் கிரந்திகுன்மம்
ஊக்கமிகு சூலைவாய் வோடுபித்தத் - தாக்குவிடம்
வீறுமோ கண்துலங்கும் வீசுபசி ரத்தமுண்டாம்
கூறுசங்கம் வேரிலை கட்கு.”

(அ.கு)

இதன் வேர்ப்பட்டைக்கு கோழை, இருமல், ஐயச்சுரம், கடுப்பு, ஐய அதைப்பு, கிரந்தி, உட்சுரம், வளிநோய்கள், வயிற்றுப்புழுக்கள் ஆகியவை போகும்.

“சங்கம்வேர்ப் பட்டை சறியிருமலைச் சுரத்தை
அங்கவா தக்கடுப்பை ஆடதைப்பைப் - பங்கமே
செய்யுங் கிரந்தியையுள் தீகால் கிருமியையிங்
வையந் தனிலொழிக்கு மால்.”

(அ.கு)

இதன் வேர்ப்பட்டைப் பாலுக்கு வீக்கம், நீரேற்றம், சுரவேகம் ஆகியவை நீங்கும்.

"சங்கம்பால் வீக்கமதைத் தான் போக்கும் நீர்வடிக்கும்.
அங்குரசு வெப்பகற்றும் ஐயமில்லை."

- (அகத்தியர் குணவாகடம்)

இலை

- வ.கு. இலையின் குடிநீரை வளிநோய்கட்கு வழங்கி வரலாம். இலையை அரைத்து அம்மைப் புண்களுக்குப் பூசக் குணமாகும்.
- இதை கரப்பானுக்கும் பூசலாம்
- இதன் இலையையும், வேப்பிலையையும் ஓர் அளவாக எடுத்தரைத்து, சிறு புன்னைக்காயளவு பிரசவமான நாள் முதல் 7 நாள் வரையில் தினம் இருவேளை கொடுத்துப் பத்தியம் வைக்க, பிள்ளைப் பெற்ற பின் உண்டாகும் அழுக்கின் தடையைப் போக்கும்.
- இதன் இலைச்சாறு கோழையை வெளிப்படுத்தும். இருமலைத் தணிக்கும்.

"சங்கதட் சூரணஞ் சாப்பிட வெருகடி
யங்கவுள் ளிரணமு மரந்தையு மறுமே"

- தேரன் வெண்பா

- சங்கம் பட்டையைப் பொடித்து முறைப்படி உட்கொண்டு வந்தால், உடலினுள் உண்டான, புண்களும் ஏனைய நோய்களும் அகலும்.

வேர்ப்பட்டை

- வேர்ப்பட்டையை இடித்துப் பிழிந்த பாலை, 1 (அ) 1 ½ பலமெடுத்து, அதற்கிருமடங்கு, வெள்ளாட்டுப்பால் சேர்த்துக் கொடுத்துவர சிறுநீரைப்பெருக்கி வெளிப்படுத்துவதுடன், உடல்வீக்கம், சுரவெப்பம் இவைகளையும் போக்கும்.
- வேர்ப்பட்டையை அரைத்துப் பூசிவர வீக்கங்கள் கரையும்.
- இதைக் குடிநீரிட்டுக் கொடுத்துவர, முறைச்சுரம், கீல்வீக்கம், கோழை, இருமல், ஐயச்சுரம், உட்சுரம் துணியும்.

- குணப்பாடம் மூலிகை

முட்சங்கன் இலைச்சேரும் வாதநோய்களுக்கான மருந்துகள்

1. ஆதண்டைத்தைலம்
2. கைகால் முடக்குக்கான சூலை 18க்கும் நிவர்த்தி

- ஆத்துமரட்சாமிர்தமென்னும் வைத்திய சாரசங்கிரகம்

3. சர்வாங்க வாதசூரணம்
4. காலருந்திராங்க எண்ணெய்
5. கன மண்டூர செந்தூரம்
6. சிவனார்வேம்பு சூரணம்
7. வளி வெப்புக் குடிநீர்

- சிகிச்சாரந்ததீபம் 2-ம்பாகம் சி.கண்ணுசாமிபிள்ளை ப.எண்.189

BOTANICAL ASPECTS

Scientific classification

Taxonomy position according to Bentham – hooker

Bentham and Hooker classified

Kingdom	:	Plantae (Plants/ Piante)
Sub kingdom	:	Tracheobionta (Vascular plants / Piante vascolari)
Azima tetracantha	:	Linn as under
Class	:	Magnolipsida
Subclass	:	Rosidae
Series	:	Bicarpellate
Order	:	Celastrales
Family	:	Salvadoraceae
Genus	:	Salvadoraceae
Species	:	Tetracantha

Distribution

It is cultivated through out india.

Identifying characters

Habit	:	Busy shrubs with shrub spices, deccan, ceylon and coromandal coast.
Leaves	:	Elliptic, rigid, pale
Flower	:	Small, Greenish white (or) yellow. unisexual in auxillary facicles.
Seed	:	1-2 seeded, white berries about ¼” in diameter.

Chemical constituents

Leaves and stem contain 3 dimeric piperioline alkaloids.

1. Azumine
2. Azcarpine
3. Carpine

Leaves contain

Feridelin, Glutinol, Lupeol and β sitosteral

Phytochemical aspect:

Whole plant parts contain

Azimine, Azcarpine, carpine

Leaves and root contain

Terpenoids

Seeds contain

Glycosides, Acyl-glycosides, quercetin, isorhamnetin, rhamnetin, rhamnazin.

All parts contain

Clucosinolates

They are hydrolyzed into thiocyanates, iso-thiocyanates

Seeds and Roots contain

-N-methoxy-3-indolylmethly-Glucosinolate.

Seed oil contain

The fatty acids one, - Myrisfic acid 0.2%, Palmitic acid 5%, Stearic acid 15%, Arachidic acid 7%, Behenic acid 2%, Linoleic acid 18%, Eicosenoic acid 21%.

Description

Azima tetracantha is a perennial shrub growing upto 3m in hot, dry riverine scrub, particularly on alluvial (or) saline soil. The plant is dioecious, erect shrub with (1-)2 spines 0.5-5cm long in each leaf axil, sometimes scandent with stems upto 8m long, branchelets are terete (or) quadrangular, glabrous to densely hairy. The leaves of the plant are elliptical in shape and are rigid, pale green colored.

The flowers are small, greenish white (or) yellow colored, unisexual in axillary fascicles.

Fruits are globular, white shiny

Seeds are compressed, circular.

Macroscopic characters:

Azima tetracantha

Leaf structures	**Nature**	**Decussately opposite**
	Shape	Blade elliptical – oblong to ovate – oblong (or) orbicular
	Dimensions	1.5-5.5cmx0.5-4.5cm
	Stipules	Absent/rudimentary
	Leaf margin	Simple and entire
	Leaf apex	Mucronate
	Leaf base	Pinnately veined with one pair of lateral veins
Flower structures	Petals shape	Linear oblong to oblong
	Length	2-4mm
	Lobes	Triangular
	Male flowers	Stamens inserted at the buse
	Female flowers	Staminoids and superior ovary
Fruit structers	Nature	Globose berry
	Dimension	0.5-1cm diameter

Microscopic characters

Leaf structures

Transverse section	-	Dorsiventral nature
Midrib	-	Flat and hemispherical
Cuticle	-	Thin, rectangular and prominent
Vascular bundle	-	Single and abanial arc shaped phloem
Sclerenchyma	-	Absent
Lamina	-	230mm thick
Trichomes	-	Absent
Abanial epidermis	-	Stomatiferrous
Epidermal tissues	-	Stomata and epidermal cells
Stomata	-	Auisocytic
Petiole (basal and upper part)	-	1.5µm diametre circular

Stem structures

Young stem	-	1.5mm thick, consists of a distinct continuous epidermis cortex, vascular cylingder and pita
Epidermal cells	-	Squarish (or) rectangular
Cuticle	-	Thick
Stomata	-	Frequently seen
Cortex	-	150mm width, consists of chlorenchyma and parenchyma
Pith	-	Wide, homogenous and parenchymatous
Vascular cylinder	-	29 discrete vascular bundles

Root bark structures

Periderm	-	No deep fissures and contains homogenous phellan cells
Pseudocortex	-	Inner to the periderm is a wide parenchymatous zone
Secondary phloem	-	It consists of collapsed and non-collapsed phloem.

Pharmacological activity

- Antimicrobial activity
- Antioxidant
- Anti inflammatory
- Analgesic
- Antipyretic
- Hepatoprotective
- Antinephrotomic
- Antiulcer

MODERN ASPECTS

Sulphur

Vernacular Name

Sanskrit	:	Gandhaka	English	:	Sulphur
Persia	:	Gogird, Gangird	Bengali	:	Gandhaka, Gandrak
			Hindi	:	Gandhaka (or)Bali
Gujarati	:	Gandhaka	Marathi	:	Gandhaka
Assamia	:	Kiburit	Kannada	:	Gandhaka
Telugu	:	Gandhakamu	Punjabi	:	Gandhka
Tamil	:	Gandhakamu	Latin	:	Brimstone
Burma	:	Kall	Malayalam	:	Baliranga
Arab	:	Kibrika			

Toxicity

Low Emicity and poses very little if any risk to human health. Sulfa drugs ie sulfonamides can cause adverse drug reactions. The most common adverse reaction is ranging from various benign rashes to life thretening stevens-Johnson syndrome"

"Sulfa allergy" is approximately only 3%

Sulphur

Sulphur is also known as brimstone (or) burning stone because it burns easily, giving a blue flame Pure, native sulphur is quickly identified from its bright yellow colour.

It typically forms crust-like deposits around the margins of hot volcanic springs smoky volcanic chimneys called fumaroles.

Extracting the sulphur involves taking advantage of sulphur's low melting point, which is only a little above the boiling point of water.

Ref: *The complete guide to Rocks & minerals.*

General properties

Alternative name	-	Sulphur
Appearance	-	Lemon yellow sintered microcrystals
		Standard atomic weight (Ar, standard) – (32.059,32.076) conventionalL 32.06
Atomic number(z)	-	16
Group	-	Group 16 (chalcogens)

Period	-	Period 3
Block	-	P- block
Element category	-	Reactive nonmetal
Electron configuration	-	(Ne)$3S^2$ $3P^4$
Electrons per shell	-	2, 8, 6

Physical properties

Phase at STP	-	Solid
Melting point	-	388.36K (115.21°C, 239.38°F)
Boiling point	-	717.8K (444.6°C, 832.3°F)
Density (near r.t)	-	alpha: 2.07g/cm3
		Beta : 1.96 g/cm3
		Gamma: 1.92g/cm3
When liquid (at M.P)	-	1.819 g/cm3
Critical point	-	1314K, 20.7MPa
Heat of fusion	-	Mono:1.727 KJ/Mol
Heat of vaporization	-	Mono: 45 KF/mol
Molar heat capacity	-	22.75 J/(mol.K)

Atomic properties

Oridation states	-	-2, -1, +1, +2, +3, +4, +5, +6
		(a stongly acidic oxide)
Electronegativity	-	Pauling scale: 2.58
Lonization energies	-	1^{st} : 999.6 KJ/mol
		2^{nd}: 2252 KJ/mol
		3^{rd} : 3357 Kg/mol
		(more)
Covalent radius	-	105±pm
Vander waals radius	-	180pm
Other properties		

Crystal structure – orthorhombic

Thermal conductivity	-	0.205 w/(m.k) (amorphous)
Electrical resistivity	-	$2x10^{15}$ Ω.m(at 20°c) (amorphous)
Magnetic ordering	-	Diamagnetic (1)
magnetic susceptibility	-	(a) 15.5.10 $^{-6}cm^3$/mol (298 k) (2)

Bulk modulus	-	7.7GPa
Mohs hardness	-	2.0
CAS number	-	7704-34-9

History

Discovery – Chinese (3) (Before 2000 BCE)

Recognized as an element by – antoine lavoisier (1777)

Clinical aspect

History (Fire and brimstone the history of melting louisiana's sulphur)

Sulphur is a non-metalic element that occurs in both combined and fee states and is distributed widely over the earth's surface. The word sulphur is latin for "burning stone" because of its comburtibility, sulphur was used for a variety of purposes at least 4,000 years ago.

Sulphur was used by pagan priests 2,000 years before the birth of christ pre-roman civilizations used burned brimstone as a medicine and used "bricks" of sulphur as fumigants, bleaching agents and incense in religious rites. Pliny (23-27 A.D) Reported that sulphur was a most singular kind of earth and an agent of great power on other substances and had "medicinal (sic) virtues". The romans used sulphur or fumes from its combustion as an insecticide and to purify a sick room and cleanse its air of evil. Homer in the odyssey reported the same uses in 1000 B.C

The Greeks and Romans discovered that sulphur could be utilized to make fire. The romans also experimented with using sulphur with tar, rosin, bitumen and other combustibles. Their work resulted in the production of incendiary weapons. Crusaders returning from the holy land in the early 1300s brought with them the knowledge of gunpowder, which had been developed by the chinese during the time of confucius by mixing sulphur with other substanes.

OCCURRANCE

1. The greatest quantity of naturaly occuring sulphur by for is combined with other elements, most notably the sulfides of copper, iron, lead and zinc and the sulfates of barium, calcium (commonly known as gypsum), magnesium and sidium.
2. Early civilization met their major needs from the easily mined native sulphur deposits near active and extinct volcanoes.

3. Archelogical investigations have revealed that the Romans obtained sulphur from Etrusan mines. Some of Pliny's writing document sulphur mining from the islands north of sicily.
4. From the late 1700s to the late 1800s 95% of the world's manufacturing needs were met by the sicilian sulphur deposits.

But monopolistic practices and high prices eventually forced industrial consumers to look for a new supply.

Industrial uses of sulphur

From a rather meager beginning, sulphur has become one of the basic materials of industrial production.

1. Sulphur is used to make gunpowder, matches, phosphate, insecticide, fungicides and medicine and in vulcanizing rubber and imporegnating wood and paper products, but these are only minor uses.

2. Nearly 90% of the domestic production is converted to sulphonic acid. This workhorse of chemistry is a major component in the manufacture of literally thousands of products, but especially fertilizer.

About one-half of the country's sulphur goes to the fertilizer industry. Sulphur and sulphuric acid one so necessary to manufacturing that their demand can be used as an accurate indication of the business activity.

Text book of inorganic chemistry

Bio-chemistry

Sulphur in the body

The mineral sulphur is a constituent of the amino acids cysteine, cysteine and methionine present in all cells of the body. It is most concentrated in the keratin of skin, hair and nails and is fundamental for the synthesis of collagen, which keps the skin elastic and yound looking.

It also is found as a part of glucosamine and chondroitin sulfate fixed in healthy bones and cartilage.

Sulphur is present in certain enzymes, hormones (Insulin, Anterior pitutory) and substances like glutrtathione, thiamine, biotin, coenzyme –A, lipoic acid and taurocholic acid.

Dietary source

It is found in protein-rich foods such as meat, poultry, fish, eggs, legumes and milk products other good sources include garlic, onions, brussels sprouts, asparagus, kale and wheat germ.

Common and optimal dosage range

Sulphur in the body, approximately 850mg/day of dietary intake is considered necessary.

The generally recommended dosage range for arthritis is upto 1000 mg per day.

Sulphur in blood

The total sulphur in blood (plasma) averages to about 3.1 mgm%

This is present in 3 distinct forms

i. Inorganic sulphate 0.5 – 1.1 mg/100
ii. Neutral sulphate 1.7 – 3.5mg /100ml
iii. Ethereal sulphate 0.1 – 1.0 mg/100ml

Absorption

Sulphur in food is ingested in 2 forms.

1. As inorganic sulphate of sodium, potassium and magnesium.
2. As organic sulphate from sulphur containing amino acids, sulpholipids, glycoproteins, chondroitin sulphate.

They are absorbed from the intestine into portal blood and reach the liver.

Metabolism

1. Most of the organic sulphur is oxidised to inorganic sulphates.
2. Unoxidised sulphur is utilized for the formation of sulphur containing substances like insulin, anterior pituitary hormone, taurocholic acid and glutathione.
3. The rest is excreted int he urine as neutral sulphur.

Detoxification

Inorganic sulphate combines in the liver with various phenolic substances produced by purefication to form ethereal sulphate, which is excreted in urine.

Excretion

Sulphur is excreted in the urine in three forms. The total amount of sulphur excreted in urine is about 1gm per day under normal condition. The excretion of sulphur varies with the intake of sulphur containing protein and the rate of tissue catabolism.

a. Inorganic sulphate – 85% of total excretion includes sulphates of sodium, potassium, clacium, magnesium.
b. Ethereal sulphate – 10% of total excretion of this is sulphate of phenol, indoxyl and skatol.
c. Neutral sulphate – 5% of total excretion. This is unoxidised sulphur. It includes cystenine, taturine, cysteine taurine, cyanites and thiocyanates.

Actions

- Its bile secretion
- Acts as laxative
- Its preaparations also act alterative, laxative, diuretic, insecticide.
- It is stimulant to the secreting organs such as skin and the bronchial mucous membranes.

- *Fundamental of biochemistry for medical students Dr.Mrs.Ambika shanmugam MBBS MSc.*

Therapeutic uses

- Skin disorders- Eczema, dry scalp, rashes, burns and abrasions
- Digestive disorders and poor liver detoxification including food allergies and indigestion.
- Arthritis – Rheumatoid and osteoarthritis.

TOXICOLOGICAL ASPECT

கந்தகம் நஞ்சுகுறிக்குணங்கள்

கொடிய நச்சுத் தன்மையுடையது அன்று. தூய்மை செய்யும் முறையிலும், செய்முறையும் சரிவரக் கவனம் பெறாத கந்தகத்தை மருந்தாக உட்கொண்டால் நாட்பட்ட காலத்தில் நஞ்சை உண்டாக்கும்.

கண்கள் மஞ்சள் நிறமாகப் பூத்திருக்கும். முகம் வெளுத்திருக்கும், உடம்பு தன் இயற்கை ஒளி குன்றி பீர்க்கம்பூப் போன்ற நிறமடைந்திருக்கும், பற்கள் கறுத்துப் பாசியடைந்து விகாரப்பட்டிருக்கும், இடைவிடாமல் வியர்வை உண்டாகும். அது மஞ்சள் நிறம் போன்ற சேற்று நீரைப் போன்றிருக்கும்.

சிறுநீர் வெள்ளாட்டு நீரைப் போன்றிருக்கும். மலம் சாமந்திப் பூவைப் போன்றும். மஞ்சள் நிறமாகயிருக்கும். வாயில் புகை நாற்றமுண்டாகும், பொய்ப்பசி, வயிற்றுவலி, வயிற்றுப்பீசம், வியர்க்குரு போன்ற பல குறிகுணங்களை உண்டாக்கும்.

முறிவு: தேவையான பொருட்கள்

- ஆவாரம் வேர், தைவேளை வேர், நீலிவேர், சுக்கு, பருத்தியிலை, சிறுநாகப்பூ ஆறு பொருட்களையும் சரியெடை எடுத்து குரிநீரிலிட்டுக் கொடுக்க கந்தகக் குற்றம் நீங்கும்.
- தாமரை வித்தை இளநீரில் அரைத்துண்ணத் தீரும்.
- மிளகு, நீலிவேர், சீரகம் இவற்றைச் சரியெடை எடுத்துக் குடிநீரிலிட்டுக் கொடுக்க கந்தக நஞ்சு முறியும்.
- மணத்தக்காளி வேர்ப்பட்டை – 10gm, அரிசித்திப்பிலி – 10gm, அதிமதுரம் - 10gm ஆகிய இவைகளைக் குடிநீரிட்டு 1 மண்டலம் (அ) நோய் நீங்கும் வரை மாலையிலும் காலையிலுமாகக் கொடுக்க கந்தக நஞ்சு முறியும்.

Sulphur toxicity causes various physiological effects in livestock such as diarrhoea, reduced growth, reproduction and loctation problems, blind staggers and death.

Some of these effects have been produced experimentally by adding. sulphate salt to the water supply. However, these physiological effects have occurred over a wide range of sulphate concentration in water.

The serum sulphate concentration is increased in presence renal functional impairment, pyloric and intestinal obstruction and leukomia.

Marked sulphate retention in advanced glomerulonephritis came the development of acidosis.

The sulphur compounds which are important from a toxicological point of

1. Sulphuric acid (Oil of vitiriol battery acid) H_2So_4
2. Sulphonamide (P-amino Benzene sulphonamide)
3. Hydrogen sulphide (Sulphuretted hydrogen)H2S
4. Sulphur-di-oxide(So_2)

1.Sulphuric acid:

Colourless, heavy, hydroscopic, oily liquid, which emits fumes, when exposed to the air. a stronger form of acid is known as phyrosulphuric acid which is a brown, oily, fuming liquid and is represented by the formula $H_2S_2O_7$.

Symptoms

Burning pain in the mouth, throat oesophagus and stomach with brownish (or) blood stained vomit.

Tongue is swollen, lips are usually swollen and excoriated.

Occassionaly hypersalivation has been observed on the second (or) third day.

Fatal dose :

5-10ml

Fatal period :

18-24 hours

2.Sulphonamide (P-amino Benzene sulphonamide)

It occurs as a white crystalline odourless substance slightly bitter taste and is relatively insoluble in water.

It is generally administered by the mouth, but may be administered hypodeomically (or) intramuscularly.

Symptoms

Abdominal pain, diarrhoea, skin eruptions, cyanosis, crystalluria, oliguria, anuria, nephrosis purpura, haemolytic anaemia, leucopenia, jaundice, delusions, delirium, encephalopathy and peripheral neuritis.

Haemolytic anaemia is sometimes due to deficiency of glucose-6-phosphate dehydrogenase activity in red blood cells.

Fatal dose:

A single dose about 10gm may cause death.

3.Hydrogen sulphide

Colourless, transparent gas, having a somewhat sickly sweetish taste and an odour of rotten eggs.

It is formed during the decomposed process of organic substances containing sulphur and may be formed as a by product in some of the sulphur industries. It is often found in large quantities in sewers (called stink dump) priry vaults and tannary rats.

Hydrogen sulphide is not a cumulative poison.

Symptoms

Loss of sense of smell, dizziness, headache, nausea, vomiting, abdominal pain, muscular prostration, tetanic convulsion, delirium, stupor, coma and death.

In largely diluted it may sometimes produce febrile symptoms somewhat resembling typhoid fever.

Fetal dose and fatal period

A concentration of 0.02 percent of hydrogen sulphide in the air is sufficient as produce local irritation in man.

0.05% gives rise to alarming symptoms if breathed for half an hour, while 0.07% is dangerous, 0.18% proves total immediately

4. Sulphur dioxide

(Sulphorous acid gas (or) sulphorous anhydride) So2.

This is formed by burning sulphur (or) certain metallic sulphides. Such as ironpyrites, in air (or) oxygen, and is a bi-product in the manufacture of sulphuric acid. Few minutes exposure to 400 p.p.m is dangerous to life.

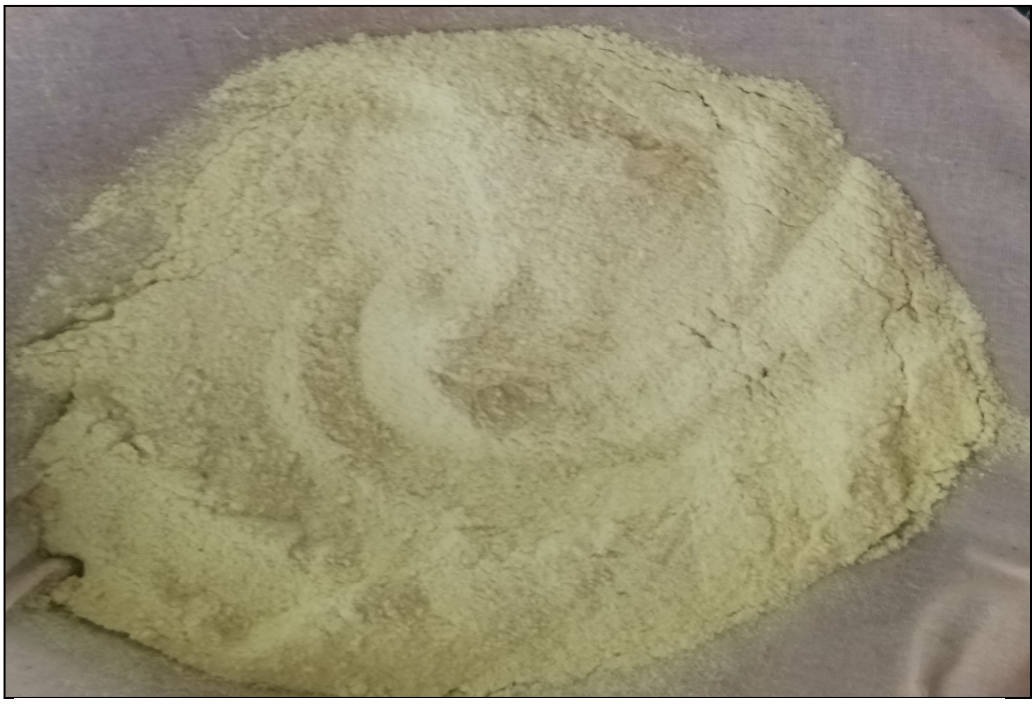
Symptoms

Inhaled in pure state, immediately coughing and sneezing accompanied by a feeling of suffocation, spasm of the glottis, dyspnoea, opacity of the cornea, cyanosis and convulsions.

It has also a remote action on the blood, causing its reduction and decomposition as shown by the formation of haematin with a brown colouration.

INGREDIENTS OF GANDHAGA MATHIRAI

Gandhagam - Before Purification



Gandhagam - After Purification

CHANGAN LEAF

GANDHAGA MATHIRAI - PREPARATION

GANDHAGA MATHIRAI - PREPARATION

GANDHAGA MATHIRAI - PREPARATION

GANDHAGA MATHIRAI - PREPARATION

GANDHAGA MATHIRAI - PREPARATION

DRUG : GANDHAGA MATHIRAI

4. MATERIALS AND METHODS

Selection of drug:

Gandhaga Mathirai mentioned in Anuboga vaidhya navaneetham (Part – 6, Pg.No. 89, Hakim P. Mohamed Abdulla Sahib) was selected for evaluating the toxic signs and symptoms when given in short and long duration.

Collection of raw drug:

The raw drugs were collected from Agricultural land in Pattukottai and Palani through proper identification.

Ingredients of GANDHAGA MATHIRAI :

- Gandhagam
- Changan leaf juice

Method of preparation:

The purified drugs were taken in the following ratio.

- Purified Gandhagam - 1 part
- Changan leaf juice - S.Q

Sufficient amount of sulphur is taken . Then it will be purified in milk into Dhumapudam 3 times. Then the sulphur is grained and taken in a ceramic bowl. Later the sulphur is completely dipped by the leaf juice of sangilai. Then it is allowed to dry in sunlight. Repeat this procedure into 30 times. Later it will be grained by kalvam. The kundrimani size of tablet is prepared and dried.

DOSAGE:

1 Mathirai

ADJUVANT:

Panaivellam

5. QUALITATIVE AND QUANTITATIVE ANALYSIS

PHYSICOCHEMICAL ANALYSIS

Sample Description : ***GANDHAGA MATHIRAI***

Equipment used : Atomic Absorption Spectrometer (AAS)

Colour:

About 50gm of ***GANDHAGA MATHIRAI*** was taken in a clean glass beaker and tested for its colour by viewing again a water opaque background under direct sunlight.

pH:

The pH of ***GANDHAGA MATHIRAI*** was estimated as per the method prescribed in Indian Standard (IS) – 6940 (1982). One gram of the ***GANDHAGA MATHIRAI*** was taken into a 100ml graduated cylinder containing about 50ml of water and filled up to the mark with water. The cylinder was stopped and shaken vigorously for two minutes and the suspension was allowed to settle for an hour at 25^0 to $27^{0.}$ About 25ml of the clear aqueous solution was transferred into a 50ml breaker and tested for pH using DIGISUN digital pH meter (DIGISUN Electronics, Hyderabad, India)

Determination of Ash Value:

Weighed accurately 2 grams of ***GANDHAGA MATHIRAI*** in tarred platinum or silica dish and incinerate at a temperature not be exceeding 450^0C until free from carbon, cooled and weighed. Calculate the percentage of ash with reference to the air dried drug.

Water Soluble Ash:

To the gooch crucible containing to the total ash, added 25ml of water and boiled for 5 minutes. Collected the insoluble matter in a sintered glass crucible or on ash less filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature nor exceeding 450^0 C subtract the weight of the insoluble matter from the weight of the ash the difference of the weight represents the water soluble ash. Calculate the percentage of water soluble ash with reference to the air dried drug.

Acid Insoluble Ash:

Boiled the ash 5 minutes with 25ml of 1:1 dil HCL. Collect the insoluble matter in gooch crucible on an ash less filter paper wash with hot water and ignite. Cooled in a desiccators and weighted calculated the percentage of acid insoluble ash with reference to the air dried drug.

Loss on Drying:

Five grams of ***GANDHAGA MATHIRAI*** is heated in a hot oven at 105^0C to constant weight and the percentage of loss of weight has calculated there from.

PHYTOCHEMICAL ANALYSIS

PROCEDURE

Test for Alkaloids (Ansari, 2006)

The extract was evaporated in a test tube. To the residue dilute HCL was added, shaken well and filtered.

1.Mayer's Test:

To the 2-3 ml of filtrate Mayer's reagent was added. Formation of yellow precipitation showed the presence of alkaloids.

Ansari, S. H. 2006.

Essentials of pharnacognosy, 1st edition, Birla publications, New Delhi. pp. 357-359, 588-590.

2.Dragendroff's Test:

To 2 mg of the ethanolic extract 5 ml of distilled water was added, 2M Hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicates the presence of alkaloids.

3.Hager's Test:

To 2 mg of the ethanolic extract taken in a test tube, a few drops of Hager's reagent was added.Formation of yellow precipitation confirms the presence of alkaloids.

Test for Carbohydrates and Glycosides

1.Molisch Test

2 mg of ethanolic extract was shaken with 10ml of water, filtered and the filtrate was concentrated. To this 2 drops of freshly prepared 20% alcoholic solution of α- naphthol was added. 2 ml of conc. sulphuric acid was added so as to form a layer below the mixture. Redviolet ring appear, indicating the presence of carbohydrates which disappear on the addition ofexcess of alkali.

2.Legal's Test

The test is employed for digitoxose containing glycosides. The extract of drug is dissolved in pyridine, sodium nitroprusside solution is added to it and made alkaline, pink or red color is produced.

3.Borntrager's Test

Borntrager's test is employed for presences of anthraquinones. The drug is boiled with dilute sulphuric acid, filtered and to the filtrate benzene, or ether or chloroform is added and shaken well. The organic layer is separated to which ammonia is added slowly. The ammoniacal layer shows pink to red color due to presences of anthraquinone glycosides.

Test for PhytoSteroids (IP, 1996)

1.Salkowski Test:

To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Indian Pharmacopoeia (IP). 1996.

Govt. of India, Ministry of Health and Family Welfare Published by the Controller of Publications, New Delhi, A-47, A-53, A-54.

2.Liebermann-Burchard's test

2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour indicates the presence of steroids.

Test for Flavanoids (Kokate, 1994)

Shinoda Test:

To the extract, 5 ml of 95% ethanol and few drops of concentrated hydrochloric acid was added. To this solution 0.5 gm of magnesium turnings were added. Pink colouration indicated the presence of flavanoids.

Kokate, C. K. 1994.

Practical Pharmacognosy, 4th edition, Vallabh Prakashan, New Delhi. 4 - 29.

Test for Tannins (Mukherjee, 2002)

Lead Acetate Test:

On addition of lead acetate solution to the extract white precipitate appeared.

Mukherjee, P. K. 2002.

Quality control of herbal drugs, business horizons pharmaceutical publishers, New Delhi. 356 - 358.

Saponin (Ansari, 2006)

Foam Test:

Drug extract was shaken vigorously with water. No persistent foam was formed.

QUANTITATIVE ANALYSIS OF GANDHAGA MATHIRAI

PROCEDURE:

Quantitative Estimation of carbohydrate

The total sugar content was estimated by Anthrone method (Roe, 1955). A known amount of the sample was taken, ground well with 80% ethanol and was centrifuged at 4000 rpm. From the supernatant, 0.5 ml was taken and 5 ml of anthrone reagent was added. The tubes were kept in a boiling water bath for 15 min. After that, they were kept in a dark room for another 15 minutes. The colour intensity developed was read in a spectrophotometer at 650 nm.

Ref: ROE, J. H. (1955), "The determination of sugar in blood and spinal fluid with anthrone reagent" Ibid., ill: 335-343.

Quantitative Estimation of flavanoids: (Evans, 1996)

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1ml of test sample and 4 ml of water were added to a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract).

Ref: Devanaboyina N et al., "Preliminary Phytochemical Screening, Quantitative Estimation And Evaluation Of Antimicrobial Activity Of Alstoniamacrophylla Stem Bark" ***IJSIT, 2013, 2(1), 31-39***

Quantitative Estimation of Saponins: (Evans, 1996)

Methanolic and water extract was dissolved in 80% methanol, 2ml of Vanilin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 600c for 10min, absorbance was measured at 544nm against reagent blank. Diosgeninis used as a standard material and compared the assay with Diosgenin equivalents.

Ref: Devanaboyina N et al., "Preliminary Phytochemical Screening, Quantitative Estimation And Evaluation Of Antimicrobial Activity Of Alstoniamacrophylla Stem Bark" ***IJSIT, 2013, 2(1), 31-39***

Quantitative Estimation of Tannins: (Robert, E.B. 1971. Agro.J.63, p.511)

1ml of the extract was mixed with 5ml of vanillin hydrochloride reagent (mix equal volumes of 8% HCL in methanol and 4% vanillin in methanol). The mixed was allowed to stand for 20mins and measure the absorbance at 500nm. The standard graph was plotted for working standard catechin solution (0 to 250µg/µl).

Ref: *Robert*, *EB*, "Method for estimation of tannin in grain sorghum ", *Agro J* , vol. *63*, *1971*,*p.511*; 10.

FOURIER TRANSFORM – INFRA RED SPECTROSCOPY

PERKIN ELMER – SPECFTRUM ONE

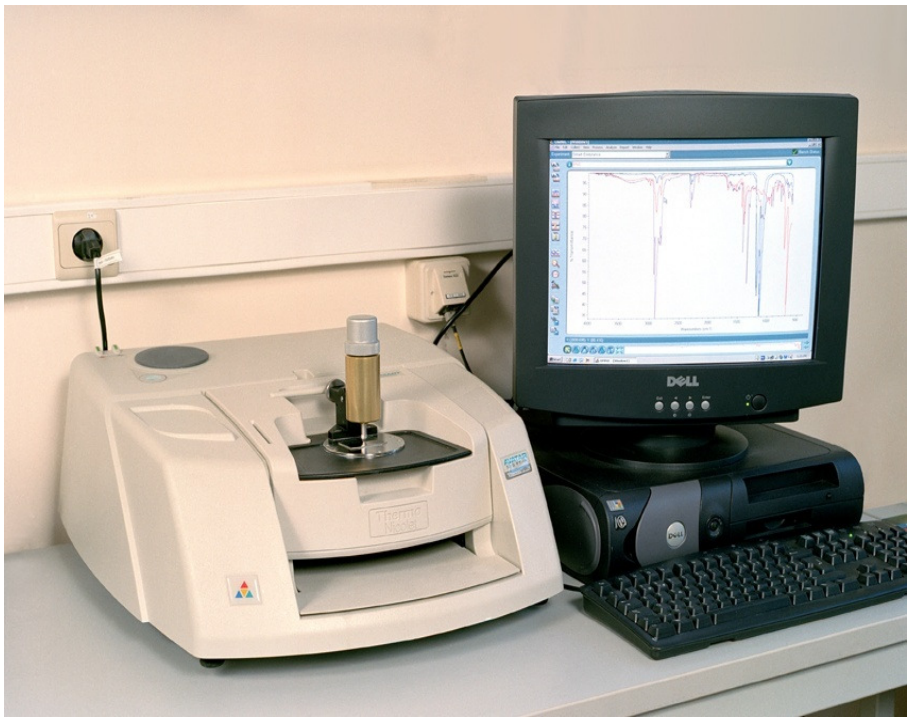
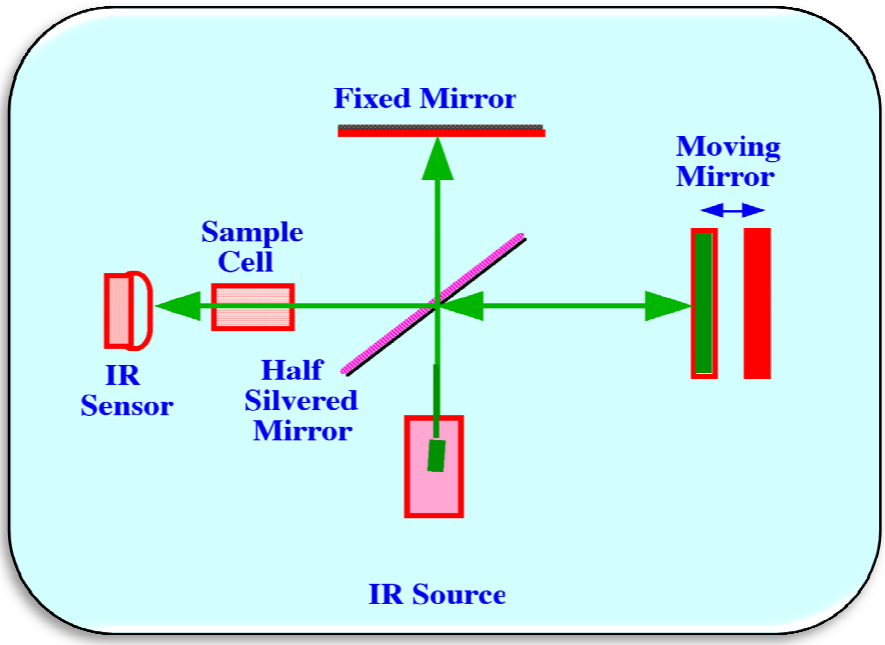


Fig. 3: FTIR Apparatus

FTIR-Mechanism



FOURIER TRANSFORM – INFRA RED SPECTROSCOPY
PERKIN ELMER – SPECFTRUM ONE

Introduction

Vibrational spectroscopy is an extremely useful tool in the elucidations of molecular structure. The spectral bands can be assigned to different vibrational modes of the molecule. The various functional groups present in the molecule can be assigned by a comparison of the spectra with characteristic functional group frequencies. As the positions of the bands are directly related to the strength of the chemical bond, a large number of investigations including intermolecular interactions, phase transitions and chemical kinetics can be carried out using this branch of spectroscopy.

In IR spectroscopy, the resonance absorption in made possible by the change in dipole moment accompanying the vibrational transition. The infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for characterization organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is carried out by using Fourier transform technique.

Principle

Infra red spectroscopy involves study of the interaction of electromagnetic radiation with matter. Due to this interaction,l electromagnetic radiation characteristic of the interacting system may be absorbed (or emitted). The experimental data consist of the nature (frequency of wave length) and the amount (intensity) of the characteristic radiation absorbed or emitted. These data are correlated with the molecurlar and electronic structure of the substance and with intra – and inter molecular interactions.

FT-IR spectroscopy is used primarily for qualitative and quantitative analysis of organic compounds, and also for determining the chemical structure of inorganic materials. The region between 500-4000 wave number is referred to as the finger print region. Absorption bands in this region are generally due to intra molecular phenomena and are highly specific for each material. The specificity if these bands allow computerized data searches to be performed against reference libraries to identify a material.

Table of Characteristic IR Absorptions

Frequency, cm-1	**Bond**	**Functional group**
3640 - 3610 (s, sh)	O-H stretch	Free hydroxyl alcohols phenols
3500 - 3200 (s,b)	O-H stretch, H – bonded	Alcohols, phenols
3400 – 3250 (m)	N – H stretch	Primary, secondary, amines, amides
3300 – 2500 (m)	O – H stretch	Carboxylic acids
3330 - 3270 (n, s)	–C (triple bond) C – H : C - H stretch	Alkynes (terminal)
3100 – 3000 (s)	C – H stretch	Aromatics
3100 – 3000 (m)	= C – H stretch	Alkenes
3000 – 2850 (m)	C – H stretch	Alkenes
2830 – 2695 (m)	H – C = 0; C –H stretch	Aldehydes
2260 - 2210 (v)	C (triple bond) N stretch	Nitriles
2260 – 2100 (w)	C (triple bond) C- stretch	Alkynes
1760 – 1665 (s)	C = 0 stretch	Carbonyls (general)
1760 – 1690 (s)	C = 0 stretch	Carboxylic acids
1750- 1735 (s)	C = 0 stretch	Esters, saturated aliphatic
1740 – 1720 (s)	C = 0 stretch	Aldehydes, saturated aliphatic
1730 – 1715 (a)	C = 0 stretch	Alpha, beta – unsaturated esters
1715 (s)	C = 0 stretch	Ketones, saturated aliphatic
1710 – 1665 (s)	C = 0 stretch	Alpha, beta – unsaturat aldehydes, ketones
1680 – 1640 (m)	-C = C -	Alkenes
1650 – 1580 (m)	N – H bend	Primary amines
1600 – 1585 (m)	C-C stretch (in – ring)	Aromatics
1550 – 1475 (s)	N – 0 asymmetric stretch	Nitro compounds
1500 – 1400 (m)	C –C stretch (in – ring)	Aromatics
1470 – 1450 (m)	N – 0 asymmetric stretch	Nitro compounds
1370 – 1350 (m)	C – H bend	Alkanes
1360 – 1290 (m)	C – H rock	Alkanes

1335 – 1250 (s)	C – N stretch	Aromatic amines
1320 – 1000 (s)	C – 0 stretch	Alcohols, carboxylic acids, esters, ethers
1300 – 1150 (m)	C – H wag (- CH2X)	Alkyl halides
1250 – 1020 (m)	C – N stretch	Aliphatic amines
1000 – 650 (s)	=C – H bend	Alkynes
950 – 910 (m)	O – H bend	Carboxylic acids
910 – 665 (s, b)	N – H wag	Primary, secondary amines
900 – 675 (s)	C – H "oop"	Aromatics
850 – 550 (m)	C – CI stretch	Alkyl halides
725 – 720 (m)	- C (triple bond) C-H : C- H bend	Alkynes
690 – 515 (m)	C - Br stretch	Alkyl halides

M = medium, w = weak, s=strong, n = narrow, b = broad, sh = sharp

Sampling techniques:

There are a variety of techniques for sample preparation depending on the physical form of the sample to be analyzed.

Solid : KBr or Nujol mull method

Liquid : CsI / TIBr Cells

Gas : Gas Cells

Experimental Procedure: Done at SAIF, IIT Madras, Chennai – 36KBr Method

- The Sample was grounded using – an agate mortar and pestle to give a very fine powder.
- The finely powder sample was mixed with about 100 mg dried KBr salt.
- The mixture was then pressed under hydraulic press using a dye to yield a transparent disc (measure about 13 mm diameter and 0.3mm in thickness), through which the beam of spectrometer passed.

HR SEM - METHODOLOGY

HR SEM-Methodology:

An SEM is essentially a high magnification microscope, which used a focused scanned collection beam to produce images of the sample, both top-down and, with the necessary sample preparation, cross-sections. The primary electron beam interacts with the sample in a number of key ways:-

Primary electrons generate low energy secondary electrons, which tend to emphasize the topographic nature of the specimen.

Primary electrons can be backscattered which produces images with a high degree of atomic number (Z) contrast.

Ionized atoms can relax by electron shell-to-shell transitions. Which lead to either X-ray emission or Auger electron ejection. The X-rays emitted are characteristic of the elements in the top few urn of the sample.

Sample Preparation:

Sample preparation can be minimal or elaborate for SEM analysis depending on the nature of the samples and the data required. Minimal preparation includes acquisition of a ***GANDHAGA MATHIRAI*** that will fit into the SEM chamber. And it should be analyzed.

INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES),

Fig. 5: ICPOES Apparatus

ICP OES METHODOLOGY:

ICP, abbreviation for Inductively Coupled Plasma, is one method of optical emission spectrometry, When plasma energy is given to an analysis sample from outside, the component elements (atoms) is excited. When the excited atoms return to low energy position, emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays, and the content of each element is determined based on the ray's intensity.

To generate plasma, first, argon gas is supplied to torch coil, and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas in ionized and plasma is generated. This plasma has high electron density and temperature (10000K) and this energy is used in the excitation –emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the center of the torch tube.

Sample preparation:

Solids cannot be analyzed directly. Such samples should be made into clear aqueous medium quantitatively. When acids are used to prepare solutions care should be taken. The concentration of the acids in the final provided solution should not be

more than 2% v/v. highly acidic and organic solutions cannot be analyzed. As a guide line weigh exactly, around 200mg of substance and dissolve in 5mL of 5% of water or aquaregia or whatever acid to make 100mL of final solution. Make proper dilutions, if necessary. Free HF should not present in the final solution to be aspirated.

Ideal concentration is around 100 ppm of the element of interest. Total dissolved solids should be not more than 0.2% w/v in the final solution Very dilute solution may not give reliable results. Each element has a detection limit. A minimum solution volume of 25 ml is necessary for analysis.

In ICP intensity of light emitted when the sample "sprayed or aspirated into an argon plasma" is measured at different wavelengths. The intensity of light at a given wavelength will be proportional to a particular elemental ion concentration. The intensity is calibrated with known standard concentration. For accurate quantitative results It is necessary to simulate the sample matrix condition with that of the standard. Each element generally will have many emission lines and the sensitivity is different for each of this wave length. When more than one element is present it is quite common that some emission lines interfere due to overlapping.

It is preferable to use plastic containers for sample handling and preserving samples for **ICP-OES** analysis. Glass containers can give problems especially when analyzing certain metal ions at low concentration.

The samples of ***GANDHAGA MATHIRAI*** was prepared.

BIOCHEMICAL ANALYSIS

BIOCHEMICAL ANALYSIS OF GANDHAGA MATHIRAI:

Preparation of extract:

5 gms of the drug was taken in a 250 ml clean beaker and 50 ml of distilled water was added to it. Then it was boiled welll for about 10minutes. Then it is allowed to cool and filtered in a 100ml volometric flask and made up to 100ml with distilled water. The extract is used for the qualitative analysis.

Qualitative analysis:

S.No.	EXPERIMENTS	OBSERVATION	INFERENCE
1.	**Test for calcium:** 2 ml 0f the above prepared extract taken in a clean test tube to this add 2 ml of 4% ammonium oxalate solution.	Formation of white colour precipitate	presence of calcium
2.	**Test for sulphate:** 2 ml of the extract is added to 5% barrium chloride solution.	Formation of white colour precipitate	Presence of sulphate.
3.	**Test for chloride:** The extract is treated with silver nitrate solution.	Formation of white colour precipitate	Presence of chloride.
4.	**Test for carbonate:** The substance is treated with concentrated HCL.	Formation of effervescence.	presence of carbonate.
5.	**Test for starch:** The extract is added with weak iodine solution.	Formation of blue colour	presence of starch.
6.	**Test for ferric iron:** The extract is acidified with glacial acetic acid and potassium ferro cyanide.	Formation of blue colour	presence of ferric iron.

7.	**Test for ferrous iron:** The extract is treated with concentrated nitric acid ammonium thiocyanide solution.	Appearance of blood red colour.	presence of ferrous iron
8.	**Test for phosphate:** The extract is treated with ammonium molybdate and concentrated nitric acid.	Formation of yellow precipitate	presence of phosphate.
9.	**Test for albumin:** The extract is treated with esbach's reagent.	Formation of yellow precipitate	presence of albumin.
10.	**Test for tannic acid:** The extract is treated with ferric chloride.	Formation of blue black precipitate.	presence of tannic acid.
11.	**Test for unsaturation:** Potassium permanganate solution is added to the extract.	It gets decolourised.	Presence of unsaturated compounds.
12.	**Test for the reducing sugar:** 5ml of benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and add 8 to 10 drops of the extract and again boil it for 2 minutes.	Colour change occurs.	Presence of reducing sugar.
13.	**Test for amino acid:** One or two drops of the extract is placed on a filter paper and dried well. After drying, 1 % ninhydrin is sprayed over the same and dried it well.	Appearance of Violet colour	Presence of amino acid.
14.	**Test for zinc:** The extract is treated with potassium ferro cyanide.	Formation of white precipitate	Presence of zinc.

6. PRECLINICAL TOXICITY STUDY

TOXICOLOGICAL STUDIES ON GANDHAGA MATHIRAI

OBJECTIVES

The aim of this study is to evaluate the toxicity of the substance GANDHAGA MATHIRAI , when administered orally to female Wistar Albino Rats with different doses, so as to provide a rational base for the evaluation of the toxicological risk to man and indicate potential target organs.

Guidelines followed:

OECD Guidelines No.423.

The experimental protocol was approved by IAEC (Institutional Animal Ethical Committee) as per the guidance of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forest, Government of India.

Study design and Controls:

1) Wistar Albino Rats in controlled age and body weight were selected.
2) GANDHAGA MATHIRAI was administered at 5mg/kg, 50mg/kg, 300mg/kg, 2000 mg/kg body weight as water as suspension along with blank.
3) The results were recorded on the day of drug administration approximately 1^{st}, 3^{rd} , 4^{th}, 24^{th} hours in post dosing further made in to observation upto 14 days.
4) The animals were kept in a clean environment with 12 hour light and 12 hour dark cycles. The air was conditioned at 22 ±3ºC and the relative humidity was maintained between 30-70% with 100% exhaust facility.

EXPERIMENTAL PROCEDURE

Animals

Female Wistar albino rats (150 – 200 gm) were used for the study. The animals were obtained from animal house, Kerala Veterinary and Animal Sciences, Mannuthy, Kerala. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30 – 70 %. A 12:12 light:dark cycle was followed. All animals were allowed to free access to water and fed with standard

commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee, Nandha college of pharmacy, Erode (688/PO/Re/S/02/CPCSEA) and were in accordance with the Institutional ethical guidelines (Proposal Number:NCP/IAEC/2018-19/20).

Test Compound

GANDHAGA MATHIRAI

Administration Procedure

Panaivellam was used as vehicle and various doses of GANDHAGA MATHIRAI were administered through oral route using gastric gavage tubes to animals by suspending in vehicle.

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Table-1 Group Numbering and Identification animals were marking on body

Group No	Animal Marking
1	Head
2	Body
3	Tail

The group no., sex of the animal and animal numbers were identified as indicated below using cage label and body marking on the animals.

Table – 2 Numbering and Identification cage label and body marking on the animals.

Cage No	Group No	Animal marking	Sex
1	I	H,B,T	Female
2	II	H,B,T	Female
3	III	H,B,T	Female
4	IV	H,B,T	Female
5	V	H,B,T	Female

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then drug was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses.

ACUTE TOXICITY STUDY

Acute Toxicity Studies

Acute toxicity studies were performed according to OECD-423 (Organization of Economic and Cooperation Development) guidelines.

Female Wister albino rats were selected by random sampling technique were employed in this study. The animals were fasted for 4 hrs with free access to water. The GANDHAGA MATHIRAI was administered orally at a dose of 5 mg/kg initially and mortality if any was observed for 3 days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher (50, 300, 1000 and 2000 mg/kg) doses of the GANDHAGA MATHIRAI were employed for further toxicity studies. The following general behaviour was also observed during the acute toxicity study (Ecobichon DJ, 1997).

3.1. Doses:

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighted and then drug was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses.

Table – 3. Doses

GROUP	DOSE
Group –I	Control
Group – II	5mg/kg
Group – III	50mg/kg
Group – IV	300mg/kg
Group – V	2000mg/kg

The test substance was administered as single dose. After single dose administration period, all animals were observed for 14 days.

General Behaviours

S.No	General Behaviour
1	Sedation
2	Hypnosis
3	Convulsion
4	Ptosis
5	Analgesia
6	Stupar Reaction
7	Motor activity
8	Muscle Relaxant
9	CNS Stimulant
10	CNS Depressant
11	Pilo Erection
12	Skin Colour
13	Lacrimation
14	Stool Consistancy

SUB-ACUTE TOXICITY STUDY

1.Objective

The objective of this 'Sub-acute toxicity study of GANDHAGA MATHIRAI on Wistar Albino Rats' was to assess the toxicological profile of the test drug when treated as a single dose. Animals should be observed for 28 days of drug administration. This study provides information on the possible health hazards likely to arise from exposure over a relatively limited period of time.

2. Test Guideline followed

OECD 407 Method – Sub-Acute Toxic Class Method (Repeated Dose 28-Day Oral Toxicity Study in Rodents)

3. Test substance Detail

Name: GANDHAGA MATHIRAI

Wistar albino rats of either sex weighing 150-200g were used in the study. The animals were divided in to 3 groups of 6 animals each. Group I served as control received Distilled water (1ml/kg). Group II, III & IV received the GANDHAGA MATHIRAI at the dose of 12.5 mg/kg , 25 mg/kg and 50mg/kg respectively.

Table 4. Animal Groupings

Groups	Drug Treatment
I	Control (1ml/kg, p.o)
II	GANDHAGA MATHIRAI (12.5mg/kg, p.o)
III	GANDHAGA MATHIRAI (25mg/kg, p.o)
IV	GANDHAGA MATHIRAI (50mg/kg, p.o)

Numbering and Identification

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Table-5 Group Numbering and Identification animals were marking on body

Group No	Animal marking
Control	H,B,T, HB,BT,HT
Low dose	H,B,T,HB,BT,HT
Middle dose	H,B,T,HB,BT,HT
High dose	H,B,T,HB,BT,HT

H-head, B-body, T-tail

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage . label and body marking on the animals:

Numbering and Identification cage label and body marking on the animals.

Cage no	Group no	Animal marking	Both Sex
1	Control	H,B,T	Male
		HB,BT,HT	Female
2	Low dose	H,B,T	Male
		HB,BT,HT	Female
3	Middle dose	H,B,T	Male
		HB,BT,HT	Female
4.	High dose	H,B,T	Male
		HB,BT,HT	Female

HB – Head Body, BT – Body Tail, HT – Head Tail

The vehicle (Panaivellam) and test drugs were administered orally, once daily for 28 days. Body weight, food intake and water intake were monitored at regular intervals. The animals were sacrificed on 29^{th} day for biochemical and histopathological studies. Prior to the sacrifice, animals were isolated in individual cages and fasted for 12 hrs, with water provided *ad libitum*.

Then, they were anaesthetized with pentobarbitone (45mg/kg, i.p) and the blood was collected by sino-orbital puncture. Blood samples for the determinations of hematological parameters (Ghai, 1995) were collected in heparinized tubes and used for the following determinations, hemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count and differential count (DC)

Non-heparinized tubes were used for serum biochemistry determinations. To obtain the serum, blood samples were placed at room temperature for approximately 30 min. Then, the tubes were centrifuged at 3000 x *g* for 10 min and the supernatants were taken for the determinations of SGPT (AST), ALT (SGOT), ALP, Creatinine, Blood urea nitrogen, Creatinine Phosphokinase and Lactate Dehydrogenase.

Estimation of AST and ALT

Activities of serum Aspartate transaminase (AST) and Alanine transaminase (ALT) were assayed by the method of Reitman and Frankel, 1957. 0.2 ml of serum with 1 ml of substrate (aspartate and α-ketoglutarate for AST; alanine and α -keto glutarate for ALT, in phosphate buffer pH 7.4) was incubated for an hour in case of AST and 30 minutes for ALT. 1 ml of DNPH solution was added to arrest the reaction and kept for 20 min in room temperature. After incubation 1 ml of 0.4N NaOH was added and absorbance was read at 540 nm. Activities expressed as IU/L.

Estimation of ALP (King, 1965)

Set up three test-tubes. Into the 1st (test), added 5 ml of the substrate solution (p-nitrophenyl phosphate in glycine/NaOH buffer) followed by 0.1 ml of serum. After 30 minutes reaction at 37^{0}C, the optical density was measured at 405 nm. Into the 2nd tube, 5 ml of substrate solution was added with 0.1 ml of serum. After mixing, the optical density was measured immediately. Into the 3rd tube, 0.1 ml of water was added with 5.0 ml of p – nitrophenol standard solution, optical density was measured.

Estimation of Blood Urea (Natelson et al., 1951)

Labeled three test-tubes as B, T and S. Into B, pipette, 0.02 ml water, into T, 0.02 ml blood and into S, 0.02 ml standard urea solution (40 mg urea in 100 ml of water). 0.1 ml of diacetyl monoxime solution and 5 ml of acid regent (Thiosemicarbazide) was added into all the test-tubes. Mixed and kept in a boiling water bath for 15 minutes. After cooling, the absorbance was read at 540 nm and concentration of urea in mg/dl was calculated.

Estimation of Serum Creatinine (Slot, 1965)

Labeled three test-tubes as B, T and S. Into B, pipetted, 2 ml of water, into T, 2 ml serum and 4 ml of water, into S, 3 ml of water and 1 ml of creatinine standard

(4mg/dl). 2 ml of ammonium sulphate and 2 ml of sodium tungstate was added in all the three test-tubes. Centrifuged and removed 3 ml of supernatant from each test tube. 1 ml of picric acid and distilled water was added to the supernatant of test tubes B, T and S. Absorbance was read at 520 nm and concentration of serum creatinine in mg/dl was calculated.

Determination of Creatine Phosphokinase

The activity of CK was estimated by the method of Rosalki (1967). CK catalyses the conversion of creatine phosphate and ADP to creatine and ATP. The ATP and glucose are converted to ADP and glucose-6-phosphate by hexokinase (HK). Glucose -6-phosphate dehydrogense (G-6-PDH) oxidizes D-glucose-6-phosphate and reduces the nicotinamide adenine dinucleotide (NAD). The rate of NADH formation, measured at 340nm, is directly proportional to serum CK activity. One ml of working reagent was added to 50 µl of test sample, mixed and incubated at 37^{o} C for 1min. After incubation, change in the optical density was measured for 3 min at an interval of 1min against blank at 340nm. The activity of creatine Phosphokinase was expressed as U/L.

Determination of Lactate Dehydrogenase

The activity of LDH was estimated by the method of Teitz,1976. The enzyme LDH is distributed in tissues particularly in heart, muscle and kidney. LDH catalyzes the oxidation of lactate to pyruvte in the presence of NAD which is subsequently reduced to NADH. The rate of NADH formation was measured at 340nm and is directly proportional to LDH activity. One ml of the working reagent was added to 10 µl of test sample, mixed and incubated at 37^{o} C for 1min. After incubation, change in the optical density was measured for 1min at an interval of 1 min against reagent blank at 340 nm. The activity of LDH was expressed as U/L.

After blood collection, the animals were sacrificed by cervical decapitation and the organs such as brain, heart, liver, spleen, kidney and testis were removed and weighed. The organs were preserved in 10% buffered formaldehyde for histopathological observations.

HISTOPATHOLOGICAL STUDIES

Anatomy of the liver was studied immediately after sacrificing the animals. A small portion was fixed in 10% neutral buffered formalin as described by Luna 14. Thin sections of 4-5µm were taken, stained with Haematoxylin and Eosin and histology was studied.

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett's *'t'* – test using graph pad version I. *P* values <0.05 were considered significantly.

7. RESULTS

QUALITATIVE AND QUANTITATIVE ANALYSIS

Table -6

Colour characters of *GANDHAGA MATHIRAI*

No	Nature of drug	Nature of colour
1	***GANDHAGA MATHIRAI***	Greenish

Table7— Physicochemical analysis of samples of *GANDHAGA MATHIRAI*

[Values are mean of three determinations ±SEM]

Parameters	Total ash	Values
Ash value	Water soluble ash	7.65±0.011
	Acid insoluble ash	0.85±0.011
Extractive value	Ethanol soluble extractive value	8.10±0.310
Loss on drying	Loss on drying at 70°C	7.20±0.240

SEM- singularity expansion method

Table-8

Particle size and pH of *GANDHAGA MATHIRAI*

S.No	Parameters	Values obtained
1	Particle size by SEM	0.5-2 μ
2	pH	7.140

PHYTO-CHEMICAL STUDY

This experimental study was taken up to qualitative analysis of Phyto-chemicals in the given sample using various test and the results are exhibited in Table

Table No 9: Incidence of various phyto-chemicals in Gandhaga Mathrai

S.No.	Name of Tests Conducted	Result Observed
Observation of Alkaloids		
1.	Mayer's Test	Negative
2.	Dragendroff's Test	Negative
3.	Hager's Test	Positive
Observation of Carbohydrates and Glycosides		
4.	Molisch Test	Positive
5.	Legal's Test	Negative
6.	Borntrager's Test for anthraquinones	Negative
Observation of Phytosterols		
7.	Liebermann – Burchard Test	Negative
8.	Salkowski Test	Negative
Observation of Flavanoids		
9.	Shinoda Test (Magnesium turnings & Hydrochloric acid)	Negative
10.	Fluorescence Test	Negative
Observation of Tannins		
11.	Ferric chloride test	Negative
12.	Potassium dichromate test	Positive
13.	Lead acetate test	Positive
14.	Millon's test	Negative
15.	Biuret test	Negative
16.	Ninhydrin test	Negative
Observation of fixed oils and fats		
17.	Spot test	Negative
18.	Saponification test	Positive

Observation of Lignin		
19.	Phloroglucinol test	Negative
Observation of Saponins		
20.	Frothing test	Negative

Note: *Positive indicates the presence of Phytochemical; Negative indicates the absence of Phytochemical*

Results

Phytochemical analysis of GANDHAGA MATHIRAI shows the presence of carbohydrates, glycosides , tannins, alkaloids, fixed oil and fats .

FOURIER TRANSFORM - INFRARED SPECTROSCOPY

IR TRACER - 100 THE NEW FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER

Chart- 1 FTIR results of Gandhaga Mathirai

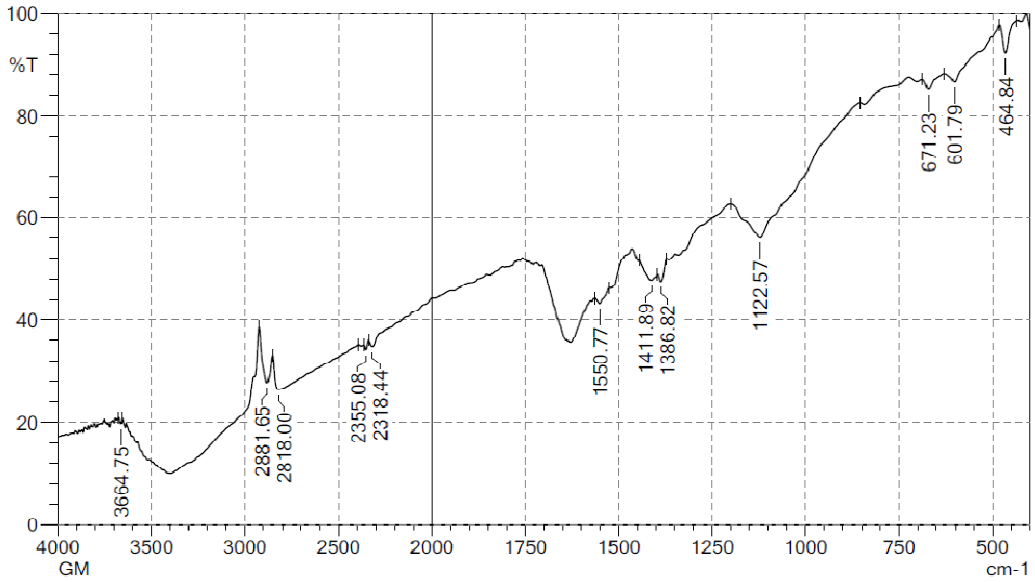


Table – 10 Functional group by FTIR study

FREQUENCY cm-1	BOND	FUNCTIONAL GROUP
464.84	C-I stretch	Aliphatic iodo compounds
601.79	C-Br stretch	Alkyl halides
671.23	C-Cl stretch	Aliphatic chloro compounds
1122.57	C-O stretch	Acyl and phenyll
1386.82	C-H rock	Alkanes
1411.89	δ=O stretch	Sulphate
1550.77	C=C stretch	Aromatic compounds
2318.44	P-H stretching	δ-lactone
2355.08	P-H stretching	Phosphines
2818.00	C-H stretching	Aldehydes
2881.65	C-H stretching	Alkanes
3664.75	O-H stretch	Water

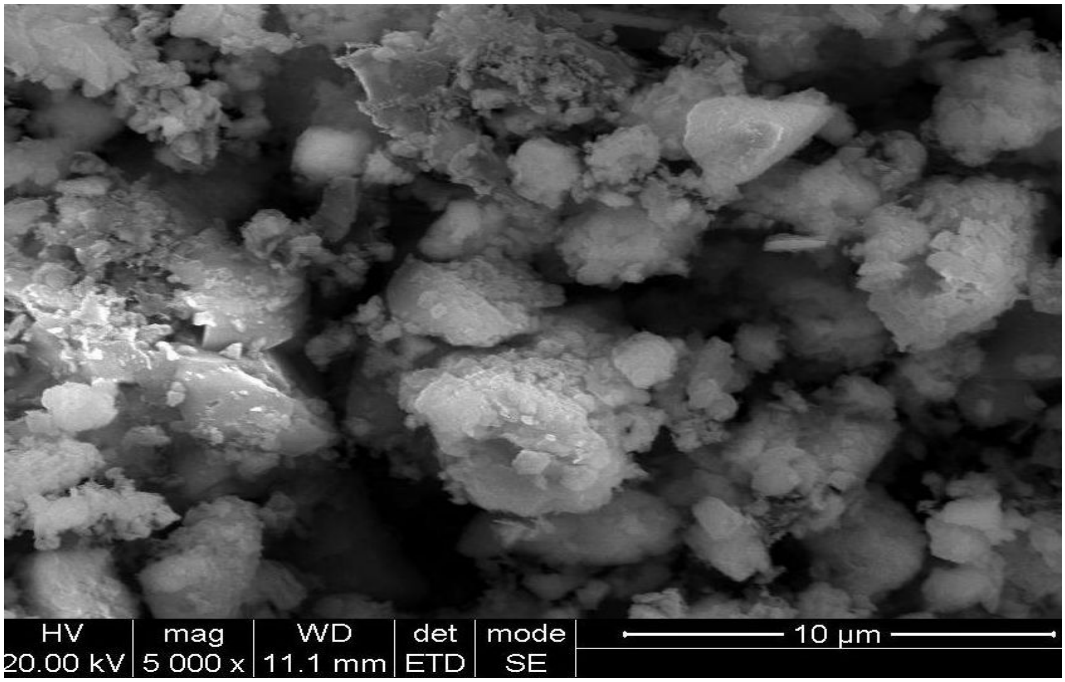
S-strong, W-weak, M-medium

Result:

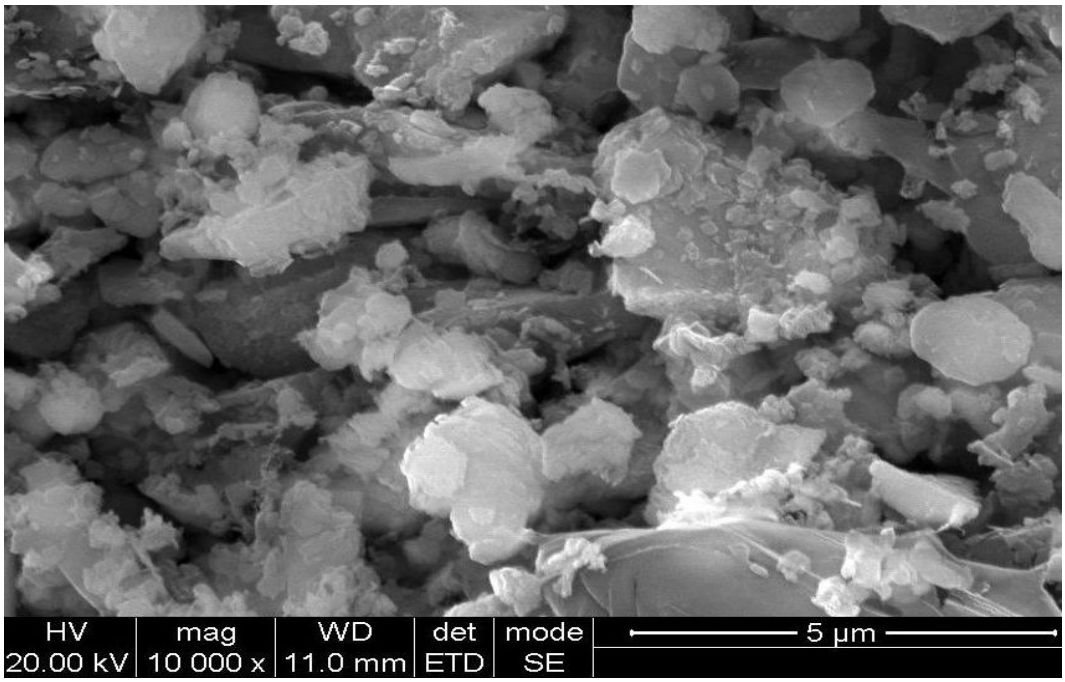
FTIR study of Gandhaga Mathirai shows the presene of functional groups such as Alkanes, Aldehydes, δ-lactone, Aromatic compounds, Acyl and phenyll, Aliphatic iodo and chloro compounds, Alkyl halides, Phosphines, Water.

SEM ANALYSIS

Scanning Electron Microscope (SEM)



SEM -5000 Magnification



SEM -10000 Magnification

Result:

The particles were stabilized and have irregular morphology. The particles were distributed in range 10 µm and the size is below 5 µm

ICP – OES of GANDHAGA MATHIRAI

ICP – OES of PP

S. no	Elements	Wavelength (nm)	Concentration
1.	Al	396.152	BDL
2.	As	188.979	BDL
3.	Ca	315.807	**21.170 mg/L**
4.	Cd	228.802	BDL
5.	Cu	327.393	BDL
6.	Fe	238.204	BDL
7.	Hg	253.652	BDL
8.	K	766.491	**23.071 mg/L**
9.	Mg	285.213	**01.104 mg/L**
10.	Na	589.592	**24.180 mg/L**
11.	Ni	231.604	BDL
12.	Pb	220.353	BDL
13.	P	213.617	**76.307 mg/L**

BDL: Below Detectable Limit(Normal-1ppm)

1% = 10000ppm,

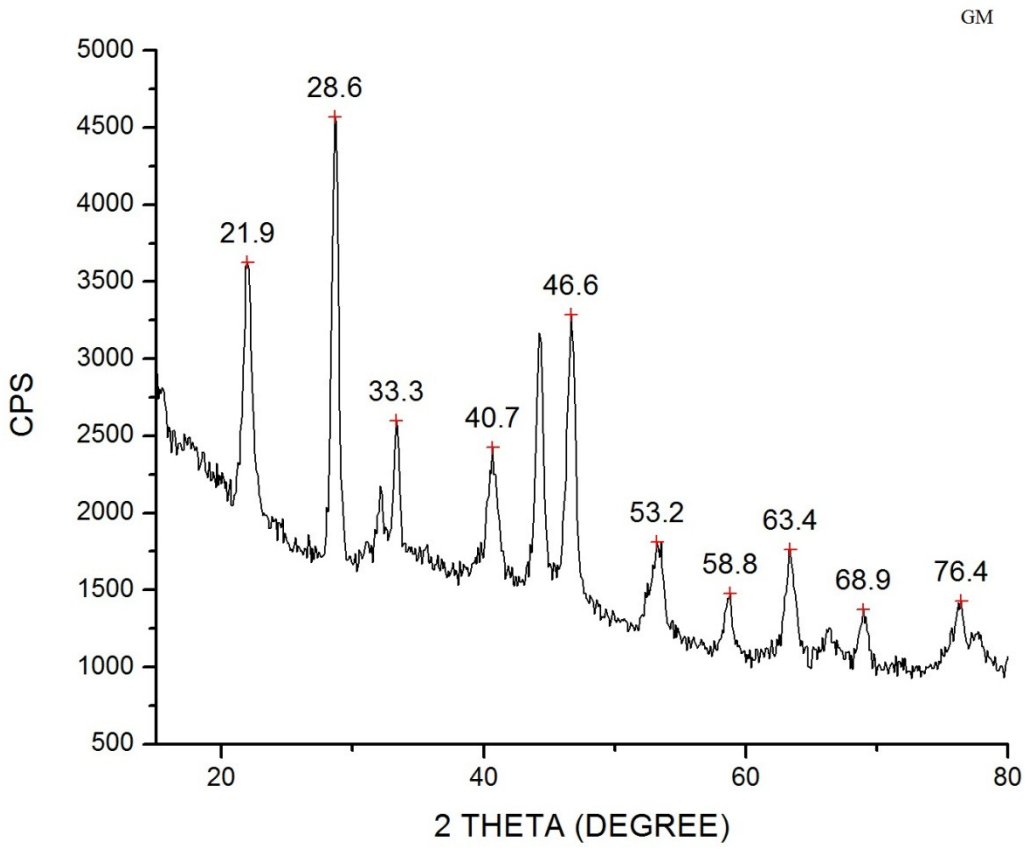
1ppm = 1/1000000 or 0.0001%

Results:

The result indicate that the formulation is extremely safe as it contains heavy metals within specified limits.

It also has physiologically important minerals like Calcium, ,Magnesium, Sodium, Phosphorus and Potassium. In *Gandhaga Mathirai,* the heavy metals like Arsenic, Mercury, Lead, Cadmium and trace element like Nikkal were below detectable level. This reveals the safety of the drug and it has free from toxic substances and has no side effects.

XRD of GANDHAGA MATHIRAI



Result:

These XRD fingerprints shows both the similarities and differences of the sample successfully and is a valuable primary tool for checking the quality control of herbo animal medicines. Modern techniques are necessary to standardize and bringout high quality herbal products owing their complex nature. The different peaks shows the presence of minerals in the sample.

BIO-CHEMICAL ANALYSIS OF *GANDHAGA MATHIRAI*

Preparation of the extract:

5 gms of the drug was taken in a 250 ml clean beaker and 50 ml of distilled water was added to it. Then it was boiled welll for about 10minutes. Then it is allowed to cool and filtered in a 100ml volometric flask and made up to 100ml with distilled water. The extract is used for the qualitative analysis.

Table - 11

BIO-CHEMICAL ANALYSIS

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**TEST FOR CALCIUM** 2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% Ammonium oxalate solution	A white precipitate is formed	**Indicates the presence of calcium**
2.	**TEST FOR SULPHATE** 2ml of the extract is added to 5% Barium chloride solution.	A white precipitate is formed	**Indicates the presence of sulphate**
3.	**TEST FOR CHLORIDE** The extract is treated with silver nitrate solution	A white precipitate is formed	**Indicates the presence of chloride**
4.	**TEST FOR CARBONATE** The substance is treated with concentrated Hcl.	No Brisk effervescence is formed	Absence of carbonate
5.	**TEST FOR STARCH** The extract is added with weak iodine solution	No Blue colour is formed	Absence of starch
6.	**TEST FOR FERRIC IRON** The extract is acidified with Glacial acetic acid and potassium ferro cyanide.	No blue colour is formed	Absence of ferric iron
7.	**TEST OF FERROUS IRON** The extract is treated with concentrated Nitric acid and Ammonium thio cyanate solution	Blood red colour is formed	**Indicates the presence of ferrous iron**

8.	**TEST FOR PHOSPHATE** The extract is treated with Ammonium Molybdate and concentrated nitric acid	No yellow precipitate is formed	Absence of phosphate
9.	**TEST FOR ALBUMIN** The extract is treated with Esbach's reagent	No Yellow precipitate is formed	Absence of Albumin
10.	**TEST FOR TANNIC ACID** The extract is treated with ferric chloride.	No Blue black precipitate is formed	Absence tannic acid
11.	**TEST FOR UNSATURATION** Potassium permanganate solution is added to the extract	It gets decolourised.	Indicates the presence of unsaturated compound
12.	**TEST FOR THE REDUCING SUGAR** 5ml of Benedict's qualitative solution is taken in a test tube and allowed to boil for 2 mts and add 8-10 drops of the extract and again boil it for 2 mts.	No colour change occurs.	Absence of Reducing sugar
13.	**TEST FOR AMINO ACID** One or two drops of the extract is placed on a filter paper and dried well. After drying, 1% Ninnhydrin is sprayed over the same and dried it well.	No Violet colour is formed	Absence of Amino acid
14.	**TEST FOR ZINC** The extract is treated with Potassium Ferrocyanide.	No white precipitate is formed	Absence of Zinc.

Inference:

Analysis reveals the presence of **Calcium, Sulphate, Chloride, Ferrous iron, Unsaturated compounds** in ***GANDHAGA MATHIRAI* .**

Biochemical Analysis report was given by **Mrs. N.Nagaprema, M.Sc., H.O.D, Bio Chemical Department, Government Siddha Medical College, Palayamkottai.**

ACUTE TOXICITY STUDY ON GANDHAGA MATHIRAI

Effect of Acute Toxicity (14 Days) of *GANDHAGA MATHIRAI*

Table 12 Physical and behavioral examinations.

Group no.	Dose(mg/kg)	Observation sign	No. of animal affected.
Group-I	Control	Normal	0 of 3
Group- II	5 mg/kg	Normal	0 of 3
Group-III	50 mg/kg	Normal	0 of 3
Group-IV	300 mg/kg	Normal	0 of 3
Group-V	2000 mg/kg	Normal	0 of 3

Table – 13 shows the effect of control – Distilled water (1ml/kg) on general behavior after single oral administration in Rat.

SL NO	GENERAL BEHAVIOR	Time of observation after control – Distilled water (1ml/kg) administration			
		1st hour	3rd hour	4th hour	24th hour
1	Sedation	-	-	-	-
2	Hypnosis	-	-	-	-
3	Convulsion	-	-	-	-
4	Ptosis	-	-	-	-
5	Analgesia	-	-	-	-
6	Stupor reaction	-	-	-	-
7	Motor activity	-	-	-	-
8	Musle relaxant	-	-	-	-
9	CNS stimulant	-	-	-	-
10	CNS depressant	-	-	-	-
11	Pilo erection	-	-	-	-
12	Skin colour changes	-	-	-	-
13	Lacrimation	-	-	-	-
14	Stool consisteny	-	-	-	-

+ Present, - Absent

Table - 14 shows the effect of GANDHAGA MATHIRAI (5mg/kg) on general behavior after single oral administration in Rat.

SL NO	GENERAL BEHAVIOUR	Time of observation after GANDHAGA MATHIRAI (5 mg / kg) administration			
		1st hour	3rd hour	4th hour	24th hour
1	Sedation	-	-	-	-
2	Hypnosis	-	-	-	-
3	Convulsion	-	-	-	-
4	Ptosis	-	-	-	-
5	Analgesia	-	-	-	-
6	Stupor reaction	-	-	-	-
7	Motor activity	-	-	-	-
8	Musle relaxant	-	-	-	-
9	CNS stimulant	-	-	-	-
10	CNS depressant	-	-	-	-
11	Pilo erection	-	-	-	-
12	Skin colour changes	-	-	-	-
13	Lacrimation	-	-	-	-
14	Stool consisteny	-	-	-	-

+ Present, - Absent

Table-15 shows the effect of GANDHAGA MATHIRAI (50mg/kg) on general behavior after single oral administration in Rat.

SL NO	GENERAL BEHAVIOR	Time of observation after GANDHAGA MATHIRAI (50 mg / kg) administration			
		1st hour	3rd hour	4th hour	24th hour
1	Sedation	-	-	-	-
2	Hypnosis	-	-	-	-
3	Convulsion	-	-	-	-
4	Ptosis	-	-	-	-
5	Analgesia	-	-	-	-
6	Stupor reaction	-	-	-	-
7	Motor activity	-	-	-	-
8	Musle relaxant	-	-	-	-
9	CNS stimulant	-	-	-	-
10	CNS depressant	-	-	-	-
11	Pilo erection	-	-	-	-
12	Skin colour changes	-	-	-	-
13	Lacrimation	-	-	-	-
14	Stool consisteny	-	-	-	-

+ Present, - Absent

Table-16 shows the effect of GANDHAGA MATHIRAI (300mg/kg) on general behavior after single oral administration in Rat.

SL NO	GENERAL BEHAVIOR	Time of observation after GANDHAGA MATHIRAI (300 mg / kg) administration			
		1st hour	3rd hour	4th hour	24th hour
1	Sedation	-	-	-	-
2	Hypnosis	-	-	-	-
3	Convulsion	-	-	-	-
4	Ptosis	-	-	-	-
5	Analgesia	-	-	-	-
6	Stupor reaction	-	-	-	-
7	Motor activity	-	-	-	-
8	Musle relaxant	-	-	-	-
9	CNS stimulant	-	-	-	-
10	CNS depressant	-	-	-	-
11	Pilo erection	-	-	-	-
12	Skin colour changes	-	-	-	-
13	Lacrimation	-	-	-	-
14	Stool consisteny	-	-	-	-

+ Present, - Absent

Table-17 shows the effect of GANDHAGA MATHIRAI (2000 mg/kg) on general behavior after single oral administration in Rat.

SL NO	GENERAL BEHAVIOR	Time of observation after GANDHAGA MATHIRAI (2000 mg / kg) administration			
		1st hour	3rd hour	4th hour	24th hour
1	Sedation	-	-	-	-
2	Hypnosis	-	-	-	-
3	Convulsion	-	-	-	-
4	Ptosis	-	-	-	-
5	Analgesia	-	-	-	-
6	Stupor reaction	-	-	-	-
7	Motor activity	-	-	-	-
8	Musle relaxant	-	-	-	-
9	CNS stimulant	-	-	-	-
10	CNS depressant	-	-	-	-
11	Pilo erection	-	-	-	-
12	Skin colour changes	-	-	-	-
13	Lacrimation	-	-	-	-
14	Stool consisteny	-	-	-	-

+ Present, - Absent

Table-18 Home cage activity

Functional and Behavioral observation	Observation	5mg/kg Group (G-I)	50mg/kg (G-II)	300mg/kg (G-III)	1000mg/kg (G-IV)	2000mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Body position	Normal	3	3	3	3	3
Respiration	Normal	3	3	3	3	3
Clonic involuntary Movement	Normal behaviour	3	3	3	3	3
Tonic involuntary Movement	Normal behaviour	3	3	3	3	3
Palpebral closure	Normal behaviour	3	3	3	3	3
Approach response	Normal	3	3	3	3	3
Touch response	Normal	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3
Tail pinch response	Normal	3	3	3	3	3

Table-19 Hand held observation

Functional and Behavioral Observation	Observation	Control	5 mg/ kg (G-I)	50 mg/kg (G-II)	300 mg/kg (G-III)	1000 mg/kg (G-IV)	2000 mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Reactivity	Normal	3	3	3	3	3	3
Handling	Normal	3	3	3	3	3	3
Palpebral closure	Normal behaviour	3	3	3	3	3	3
Lacrimation	Normal behaviour	3	3	3	3	3	3
Salivation	Normal behaviour	3	3	3	3	3	3
Piloerection	Normal behaviour	3	3	3	3	3	3
Pupillary reflex	Normal	3	3	3	3	3	3
Abdominal tone	Normal	3	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3	3

Table-20 Mortality

Group no	Dose no(mg/kg)	Mortality
Group-I	Control	0 of 3
Group-II	5 (mg/kg)	0 of 3
Group-III	50 (mg/kg)	0 of 3
Group-IV	300 (mg/kg)	0 of 3
Group-V	2000(mg/kg)	0 of 3

Results:

The results of acute toxicity study of Gandhaga Mathirai were shown on table 17. Gandhaga Mathirai didn't show any change in general behavior, toxic symptoms and did not produce mortality after 1hr to 24 hrs of oral administration. From the dose administrated in acute toxicity study, there are 3 doses 12.5, 25 and 50mg/kg were selected for further sub-acute toxicity study.

SUB-ACUTE TOXICITY STUDY

Table 21. Effect of Gandhaga Mathirai on body weight during 28 days drug administration in rats

Groups	Drug Treatment	Body Weight (gms)				
		1st Day	7th Day	14th Day	21st Day	28th Day
I	Control Distilled water (1ml/kg, p.o)	168.73± 3.42	176.22± 4.97	196.34± 4.77	210.54± 6.04	220.22± 4.40
II	**Gandhaga Mathirai** (12.5mg/kg, p.o)	186.66± 9.82	195.80± 6.77	204.33± 6.65	218.33± 7.41	226.54± 6.35
III	**Gandhaga Mathirai** (25mg/kg, p.o)	181.66± 7.92	190.83± 6.50	206.66± 7.48	221.60± 5.71	232.50± 4.22
IV	**Gandhaga Mathirai** (50mg/kg, p.o)	172.50± 4.64	189.16± 5.25	198.35± 6.95	209.65± 7.26	221.68± 6.61

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 1. Effect of Gandhaga Mathirai on body weight during 28 days drug administration in rats

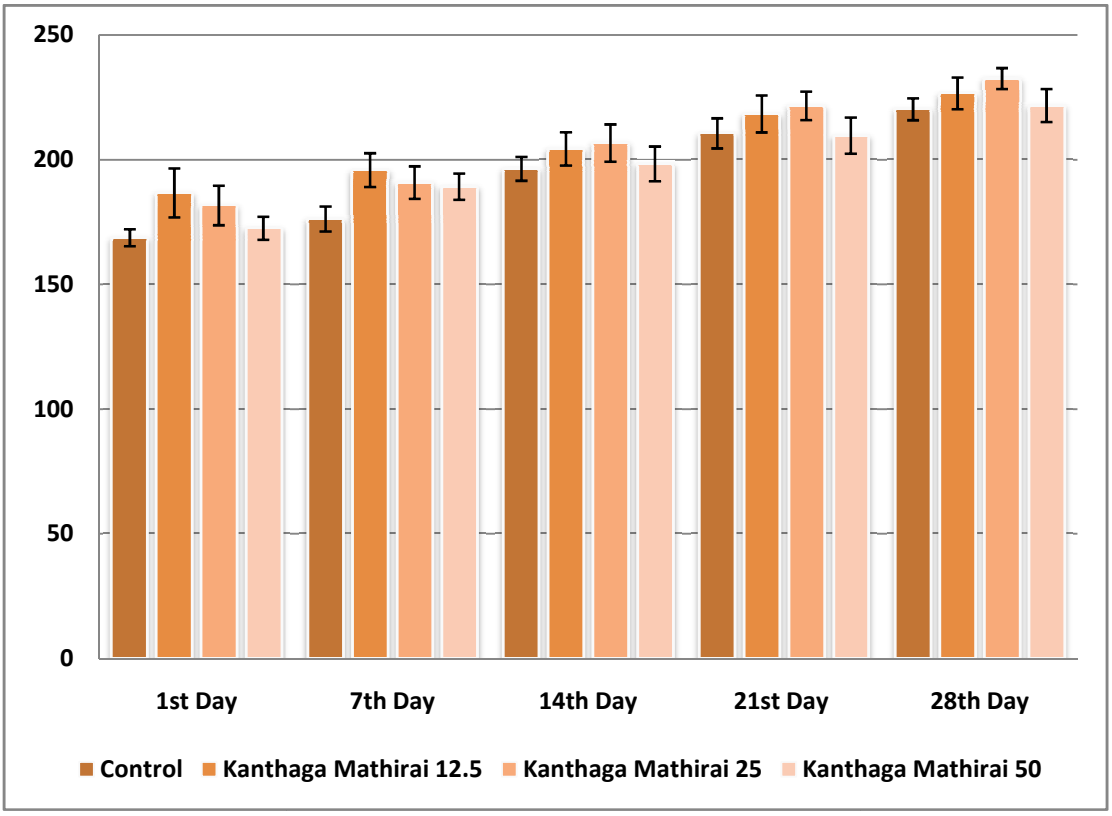


Table 22. Effect of Gandhaga Mathirai on food intake during 28 days drug administration in rats

Groups	Drug Treatment	Food Intake (gms)				
		1st Day	7th Day	14th Day	21st Day	28th Day
I	Control Distilled water (1ml/kg, p.o)	38.42±1.32	42.56±2.86	39.30±2.20	53.75±3.19	56.90±2.72
II	**Gandhaga Mathirai** (12.5mg/kg, p.o)	47.55±2.75	50.65±3.33	50.98±2.90	58.96±2.06	62.58±3.24
III	**Gandhaga Mathirai** (25mg/kg, p.o)	50.80±2.35	65.76±4.95	55.45±3.77	60.60±3.90	62.76±3.07
IV	**Gandhaga Mathirai** (50mg/kg, p.o)	56.85±2.90	55.22±3.24	62.75±2.25	66.74±3.22	65.20±4.95

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 2. Effect of Gandhaga Mathirai on food intake during 28 days drug administration in rats

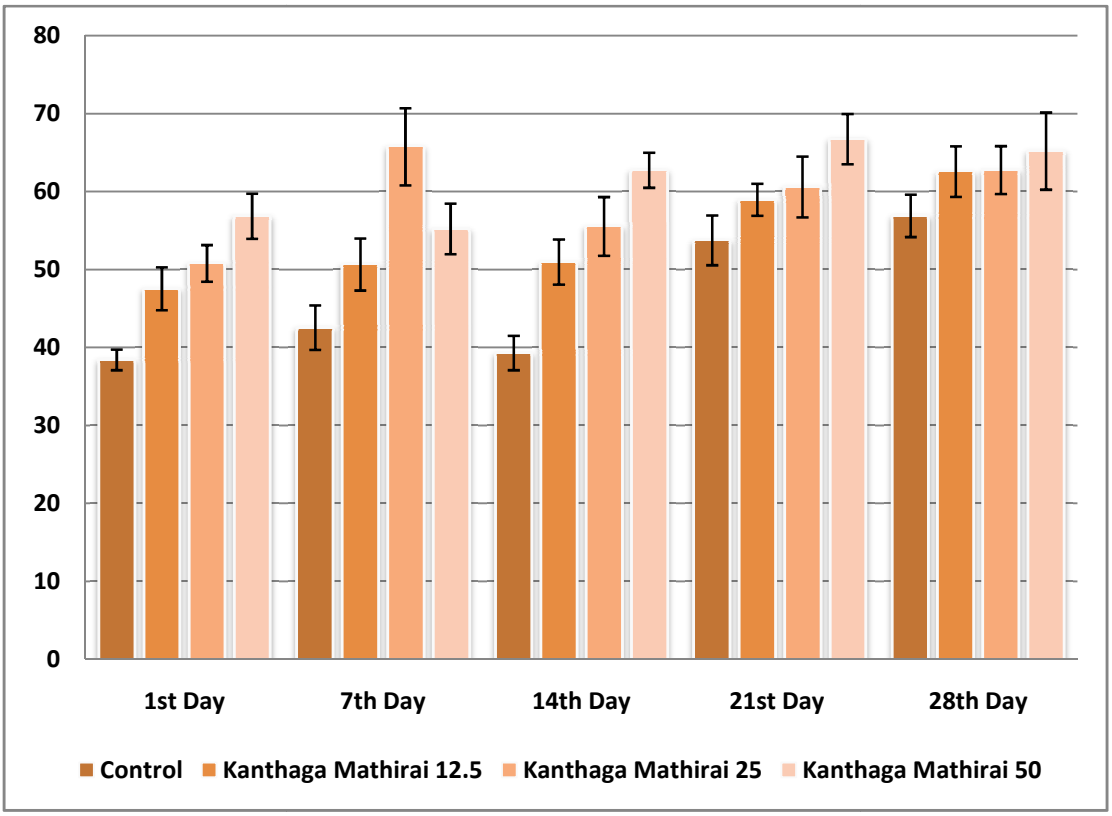


Table 23. Effect of Gandhaga Mathirai on water intake during 28 days drug administration in rats

Groups	Drug Treatment	Water Intake (ml)				
		1st Day	7th Day	14th Day	21st Day	28th Day
I	Control Distilled water (1ml/kg, p.o)	51.66±2.02	58.76±4.22	64.00±3.90	70.34±4.73	65.76±4.80
II	**Gandhaga Mathirai** (12.5mg/kg, p.o)	57.75±3.45	56.24±4.46	54.22±3.08	52.24±3.25	60.56±3.92
III	**Gandhaga Mathirai** (25mg/kg, p.o)	45.22±3.24	52.05±3.48	56.20±4.05	55.75±3.90	59.24±4.10
IV	**Gandhaga Mathirai** (50mg/kg, p.o)	54.90±3.07	53.76±4.95	56.85±4.90	58.43±4.44	57.72±3.27

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 3. Effect of Gandhaga Mathirai on water intake during 28 days drug administration in rats

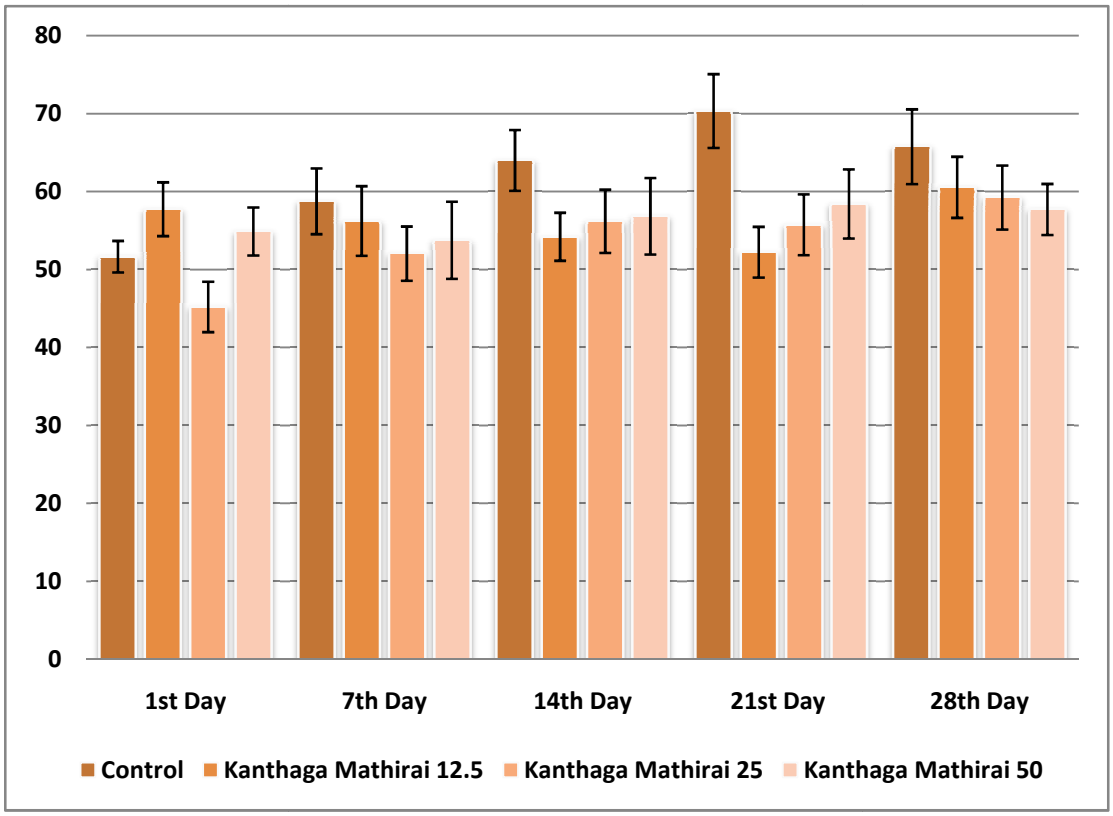


Table 24. Shows the effect of Gandhaga Mathirai on RBC, WBC and Hb in rats after 28 days drug administration

Groups	Drug Treatment	RBC million cells/cmm	WBC cells/cmm	Haemoglobin gm %
I	Control Distilled water (1ml/kg, p.o)	4.57 ± 0.16	8638.52± 87.66	11.77± 0.28
II	**Gandhaga Mathirai** (12.5mg/kg, p.o)	4.58 ± 0.15	8287.08± 241.17	11.64± 0.28
III	**Gandhaga Mathirai** (25mg/kg, p.o)	4.43 ± 0.27	8869.84± 80.65	11.93± 0.15
IV	**Gandhaga Mathirai** (50mg/kg, p.o)	4.39 ± 0.20	9278.43± 175.12*	12.24± 0.21*

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 4. Shows the effect of Gandhaga Mathirai on RBC and Hb in rats after 28 days drug administration

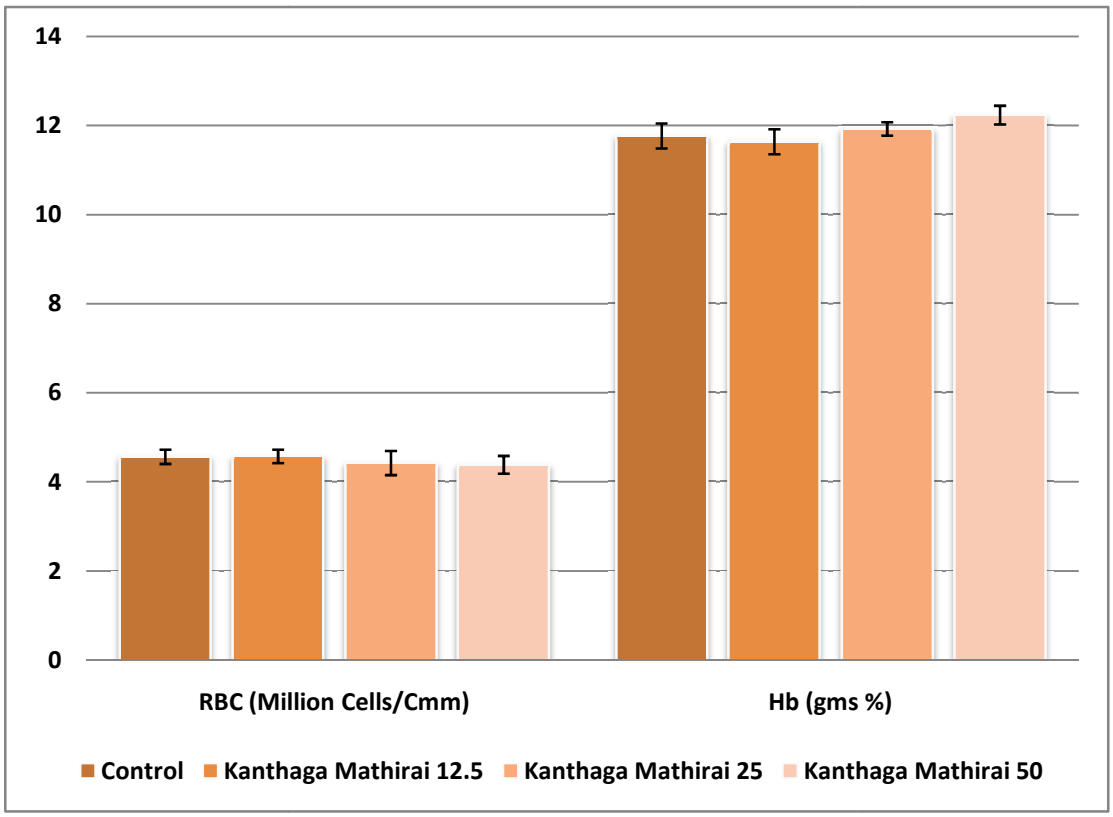


Figure 5. Shows the effect of Gandhaga Mathirai on WBC in rats after 28 days drug administration

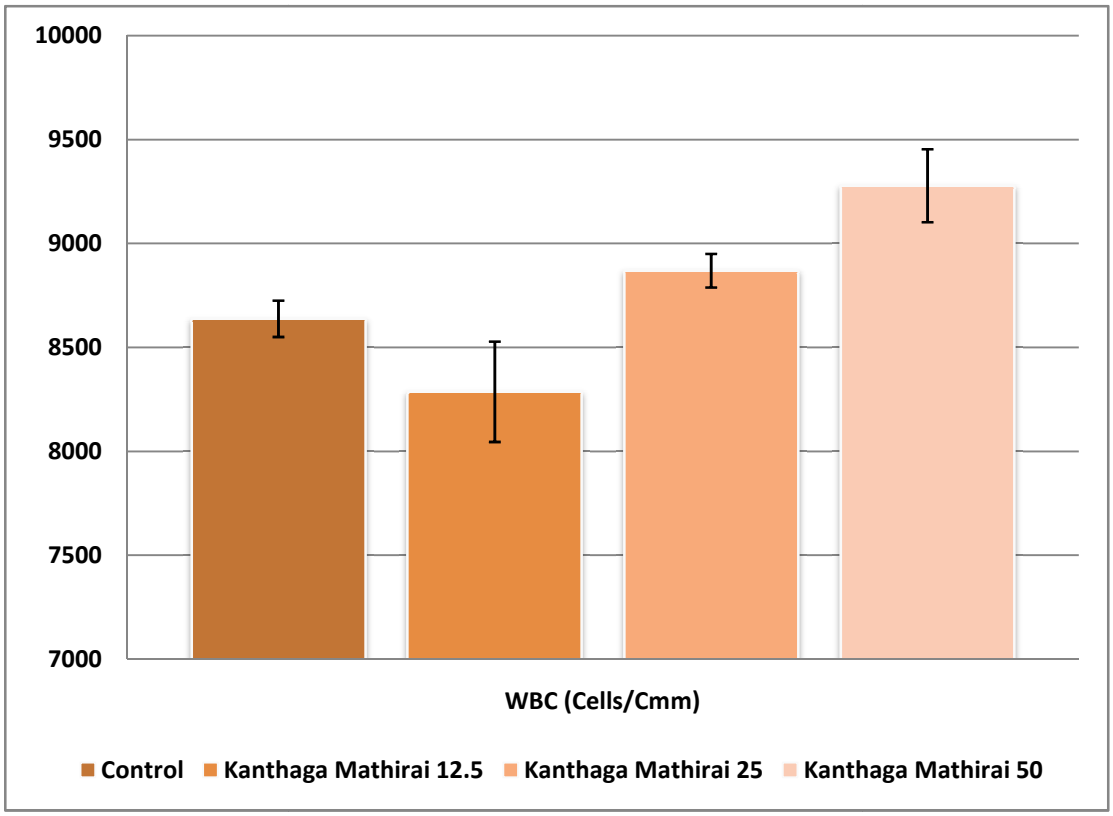


Table 24 (a) Shows the effect of Gandhaga Mathirai on Differential Count in rats after 28 days drug administration

Groups	Drug Treatment	Differential Count %			
		Neutophils	***Eosinophils***	***Monocyte***	***Lympocyte***
I	Control Distilled water (1ml/kg, p.o)	30.00± 1.79	1.17± 0.31	3.33± 0.42	65.83± 1.64
II	**Gandhaga Mathirai** (12.5mg/kg, p.o)	29.0± 0.71	1.40± 0.25	3.20± 0.20	67.40± 2.14
III	**Gandhaga Mathirai** (25mg/kg, p.o)	34.40± 0.68	1.60± 0.24	3.40± 0.24	61.80± 2.06
IV	**Gandhaga Mathirai** (50mg/kg, p.o)	30.00± 1.00	1.17± 0.17	3.17± 0.17	66.67± 1.94

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 6. Shows the effect of Gandhaga Mathirai on Differential Counts in rats after 28 days drug administration

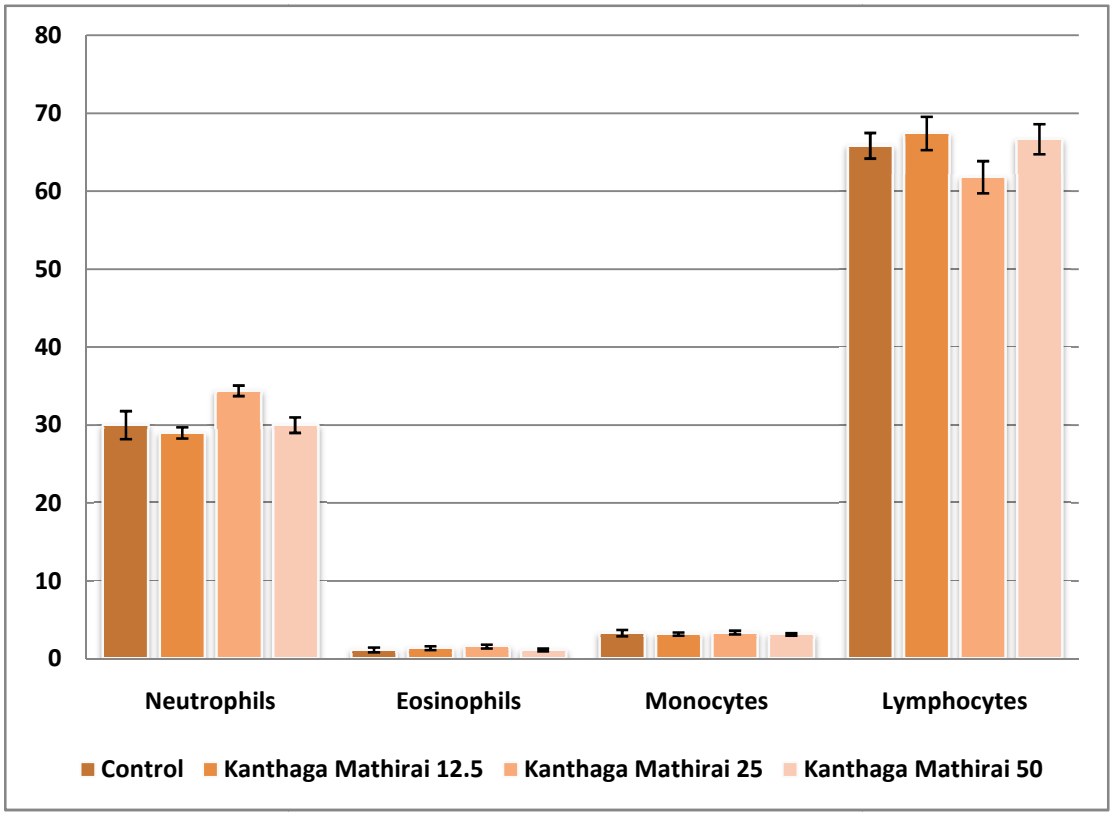


Table 25. Shows the effect of Gandhaga Mathirai on Hepatic Functions (SGPT, SGOT and ALP) in rats after 28 days drug administration

Groups	Drug Treatment	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)
I	Control Distilled water (1ml/kg, p.o)	83.83± 1.42	150.17± 4.59	270.83± 4.17
II	**Gandhaga Mathirai** (12.5mg/kg, p.o)	83.20± 1.16	147.60± 4.34	274.40± 5.90
III	**Gandhaga Mathirai** (25mg/kg, p.o)	89.40± 2.91	148.60± 4.35	286.40± 3.20
IV	**Gandhaga Mathirai** (50mg/kg, p.o)	107.33± 4.46*	185.00± 5.03*	303.67± 6.08*

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 7. Shows the effect of Gandhaga Mathirai on Hepatic Functions in rats after 28 days drug administration

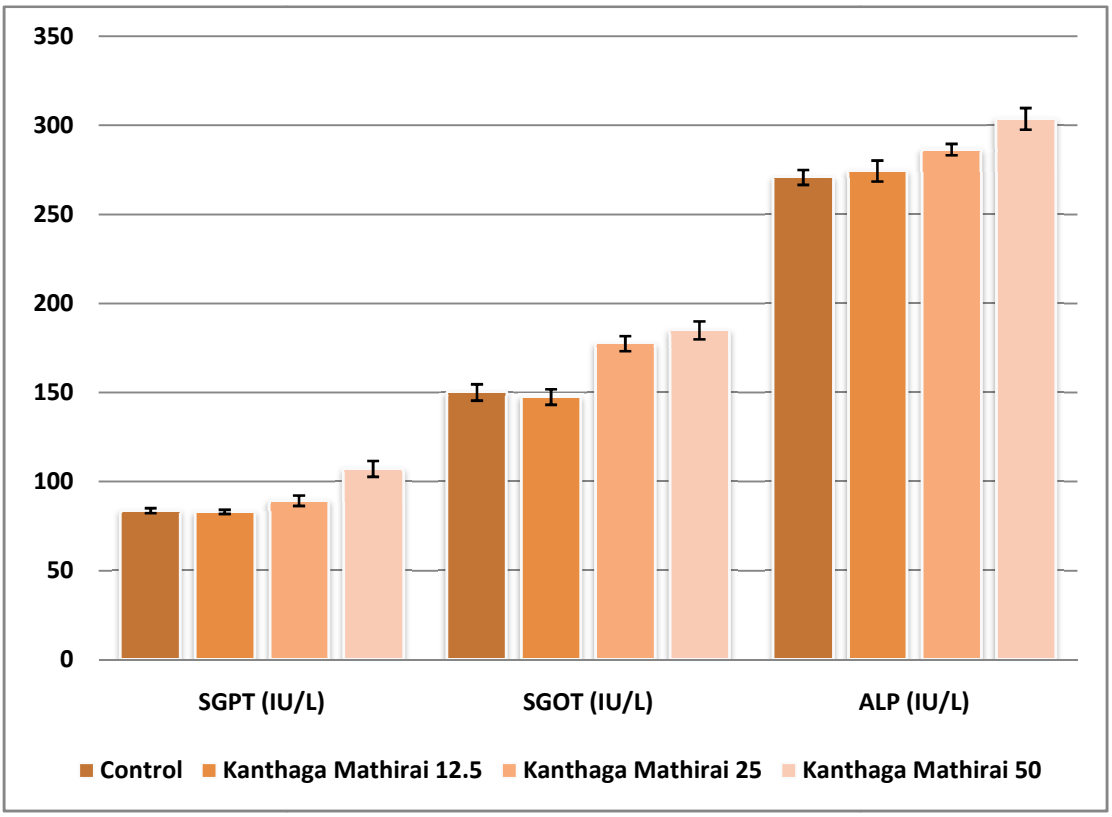


Table 25 (a) Shows the effect of Gandhaga Mathirai on Kidney Functions in rats after 28 days drug administration

Groups	Drug Treatment	Urea (mg/dl)	Creatinine (mg/dl)
I	Control Distilled water (1ml/kg, p.o)	34.67± 1.12	0.84± 0.07
II	**Gandhaga Mathirai** (12.5mg/kg, p.o)	32.80± 1.39	1.00± 0.12
III	**Gandhaga Mathirai** (25mg/kg, p.o)	36.00± 2.03	0.97± 0.08
IV	**Gandhaga Mathirai** (50mg/kg, p.o)	54.83± 1.49*	2.41± 0.11**

Values are in mean ± SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 8. Shows the effect of Gandhaga Mathirai on Kidney Functions (Blood Urea and Creatinine) in rats after 28 days drug administration

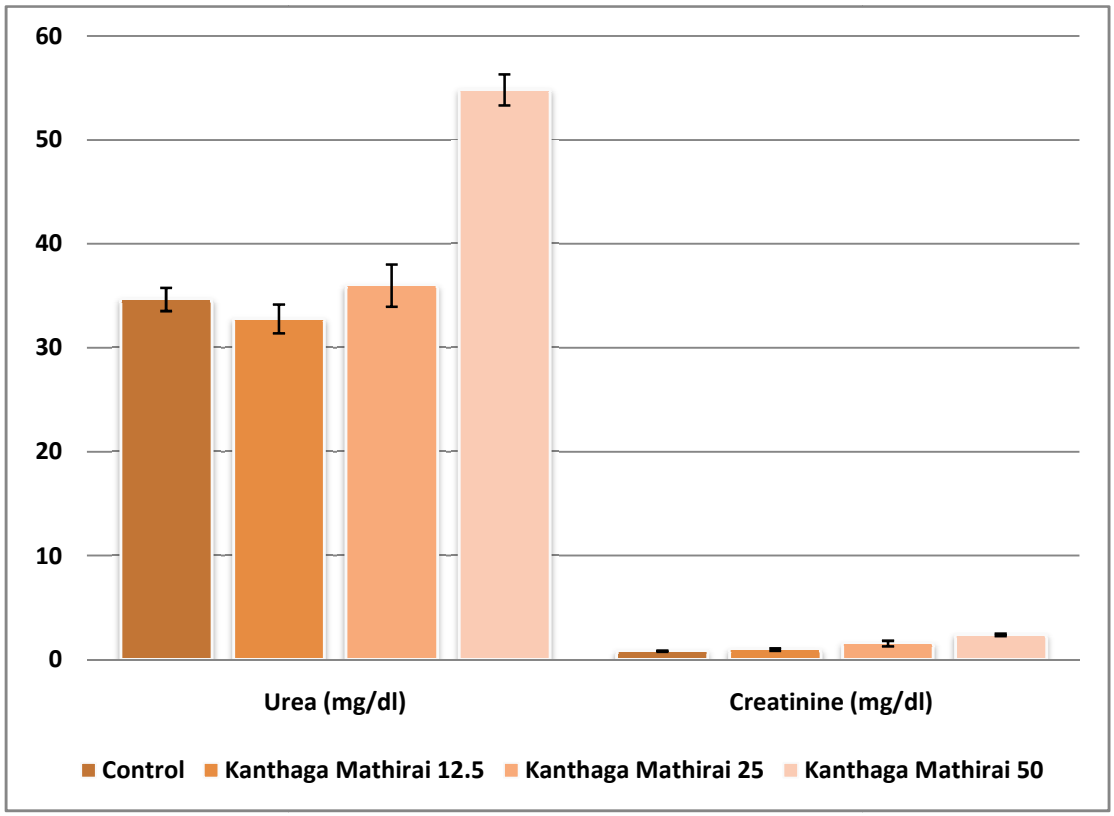


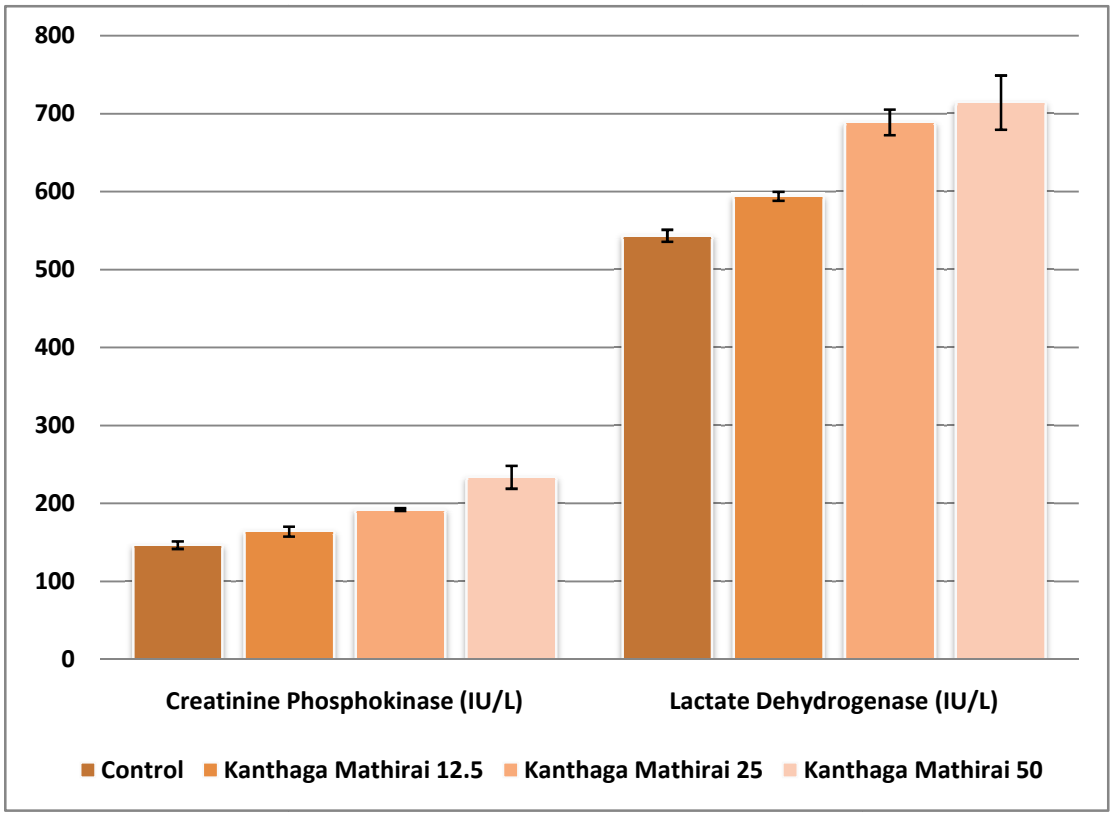
Table 25 (b)Shows the effect of Gandhaga Mathirai on Cardiac Functions in rats after 28 days drug administration

Groups	Drug Treatment	Creatinine Phosphokinase (IU/L)	Lactate Dehydrogenase (IU/L)
I	Control Distilled water (1ml/kg, p.o)	146.83± 4.79	543.50± 7.72
II	**Gandhaga Mathirai** (12.5mg/kg, p.o)	164.20± 6.34	594.20± 5.65
III	**Gandhaga Mathirai** (25mg/kg, p.o)	165.20± 6.36	597.20± 5.79
IV	**Gandhaga Mathirai** (50mg/kg, p.o)	233.83± 14.61**	714.33± 34.85**

Values are in mean ± SEM (n=6)

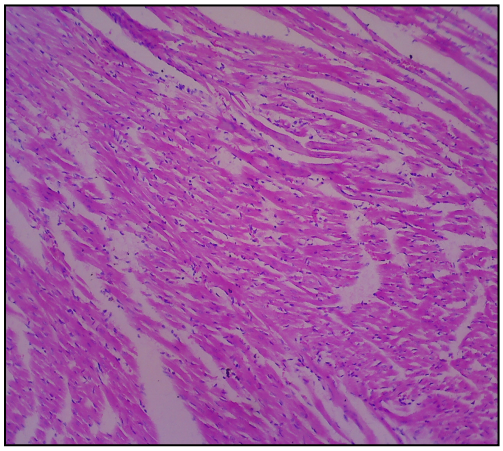
*P<0.05, **P<0.01, ***P<0.001 Vs Control

Figure 9. Shows the effect of Gandhaga Mathirai on Cardiac Functions (Creatinie Phosphokinase and Lactate Dehydrogenase and in rats after 28 days drug administration

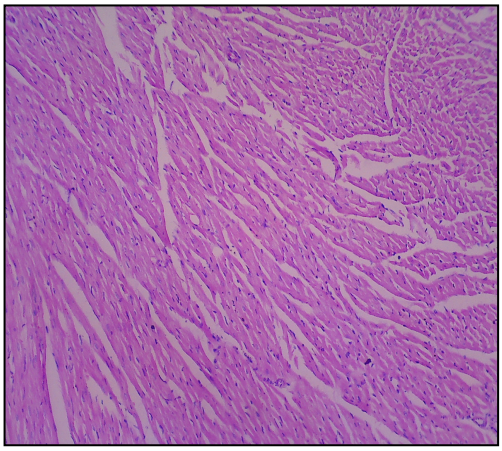


HISTOPATHOLOGICAL STUDIES

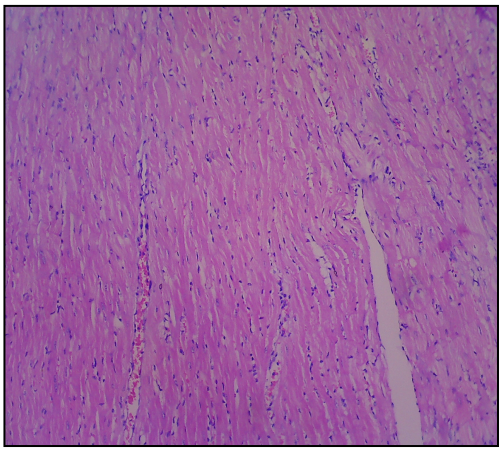
HEART



CONTROL



MIDDLE DOSE (25mg/kg)

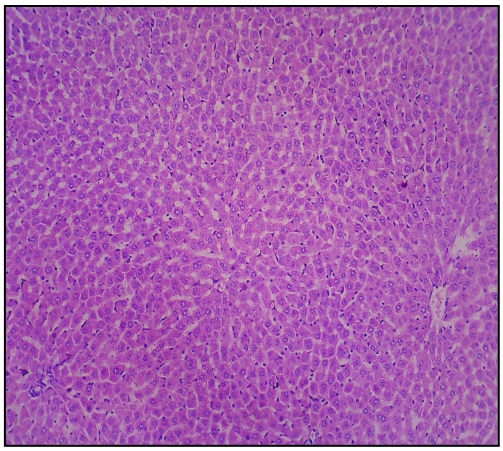


HIGH DOSE (50mg/kg)

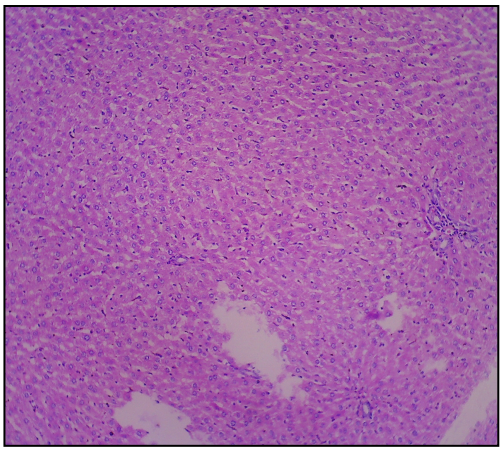
RESULT :

Normal cardiac muscle fibers seen in middle dose, it shows myocardium with myocytes showing mild inflammatory infiltrates seen in High dose.

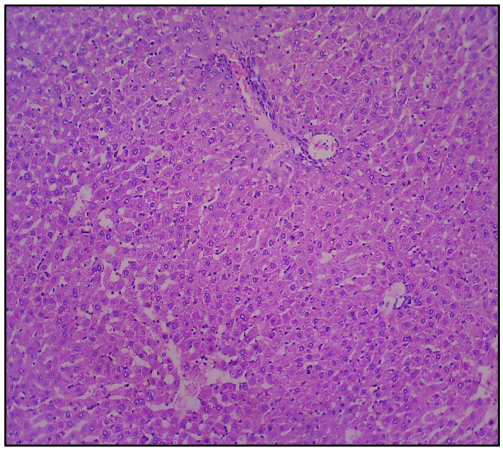
LIVER



CONTROL



MIDDLE DOSE (25mg/kg)

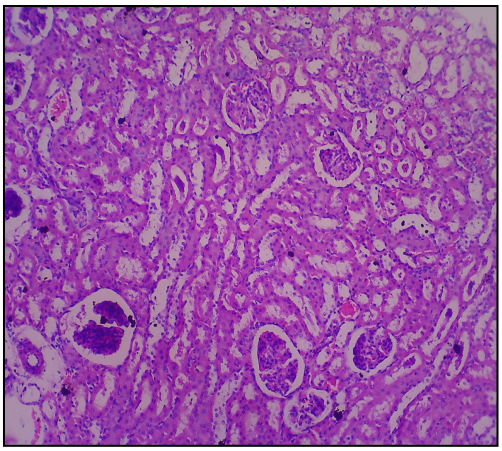


HIGH DOSE (50mg/kg)

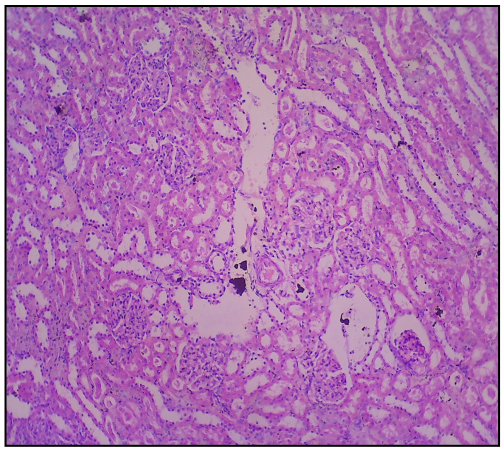
RESULT :

Normal Liver parenchyma seen in Middle dose, individual hepatocytes show no significant pathology. Portal traid shows mild inflammation seen in highdose.

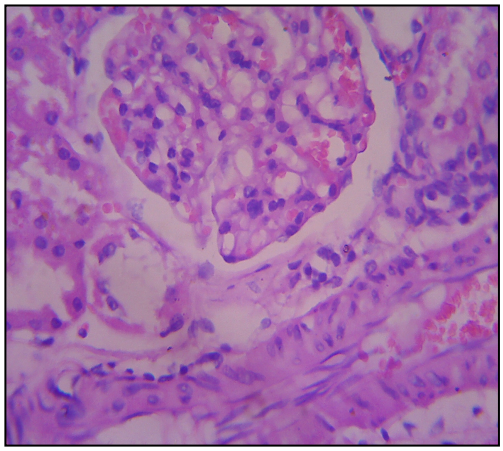
KIDNEY



CONTROL



MIDDLE DOSE (25mg/kg)



HIGH DOSE (50mg/kg)

RESULT :

Normal Renal parenchyma seen in Middle dose, blood vessels show congestion seen in highdose

RESULTS

In sub-acute toxicity study, body weight, food intake and water intake were observed on 1st, 7th, 14th 21st and 28th day of Gandhaga Mathirai after drug administration. The effect of Gandhaga Mathirai regarding body weight during 28 days treatment in rats was given in table 21 and figure 1. There was no significant change in the body weight compared to control with all the four doses of Gandhaga Mathirai during 28 days treatment.

The effect of Gandhaga Mathirai on food intake during 28 days treatment in rats was given in table 22 and figure 2. Gandhaga Mathirai did not alter the food intake at all the three dose levels as compared to control during the 28 days treatment. It indicates that it does not influence food intake.

The effect of Gandhaga Mathirai on water intake during 28 days treatment in rats was given in table 23 and figure 3. Gandhaga Mathirai did not alter the water intake at all the three dose levels tested in animals compared to control animals during the 28 days treatment. There was no significant change in water intake as compared to control.

Table 24, figure 4 and 5, shows the effect of Gandhaga Mathirai on haematological parameters like RBC, WBC and Hb in rats after 28 days treatment. 12.5 and 25 mg/kg of Gandhaga Mathirai did not produce any significant change in RBC, WBC and Hb compared to control. Gandhaga Mathirai at 50mg/kg did not alter the RBC levels compared to control, but has significant ($P<0.05$) increase in the levels of WBC and Hb compared to control.

The effect of Gandhaga Mathirai on Differential Count in rats after 28 days treatment was shown on table 24(a) and figure 6. All the three doses of Gandhaga Mathirai did not show any significant change in differential counts like Neutrophils, Eosinpophils, Monocyte and Lympocytes. From the effect of Gandhaga Mathirai on hematological parameters it was found that 12.5 and 25 mg/kg does not produce any toxicity in haemopoietic system, but high dose i.e. 50 mg/kg of Gandhaga Mathirai slightly increased the levels of both WBC and Hb.

The effect of Gandhaga Mathirai on hepatic functions in rats after 28 days treatment was shown on table 25 and figure 7. The hepatic enzymes (SGPT, SGOT and ALP) were remaining normal with low dose of Gandhaga Mathirai (12.5mg/kg). the mid dose of Gandhaga Mathirai (25mg/kg) did not alter the levels of SGPT, SGOT and ALP.

The effect of Gandhaga Mathirai on renal functions in rats after 28 days treatment was shown on table 25(a) and figure 8. Low dose and Middle dose (12.5 mg/kg and 25mg/kg) of Gandhaga Mathirai does not showed any significant change in urea and creatinine after 28 days treatment compared to control. But the higher dose 50mg/kg of Gandhaga Mathirai significantly increased the urea ($P<0.05$) and creatinine ($P<0.01$) compare to control.

The effect of Gandhaga Mathirai on Cardiac functions in rats after 28 days treatment was shown on table 25 (b) and figure 9. Low dose and Middle dose of Gandhaga Mathirai (12.5mg/kg and 25mg/kg) did not alter the cardiac biomarker enzymes as compared to control animals. The higher dose of Gandhaga Mathirai (50mg/kg) significantly ($P<0.01$) increase the levels of both Creatinie Phosphokinase and Lactate Dehydrogenase after 28 days administration, compared to control.

BIOSTATISTICAL ASPECTS

Biological assay refers to assessment of the potency of vitamins, hormones, toxicants and drugs of all types by means of the responses produced when doses are given to experimental animals. In every dose response situation, two components must be considered; the stimulus and the subject.

The stimulus is applied to the subject as a started dose namely concentration, weight, time or appropriate measure. The subject manifest a response, the level of intensity below which the response does not occur & above which the response occur, suh a value has often been called threshold. But the term tolerance is now widely accepted.

MEDIAN EFFECTIVE DOSE (E.D.50)

It is the dose which produces the desired response in half the animal population tested.

MEDIAN EFFECTIVE DOSE (E.D.50)

It is the dose which kills half the population of the animal tested.

LD50 Measurement (Toxicity)

- If the test compound shows any pharmacological activity then the L.D.50 of the drug is determined.
- By determining the L.D.50, we can justify whether to proceed with the drug or not.

Table- 26 Acute toxicity study analysis

Group	Dose in mg/kg	No. of rats	No. of rats died
I	Distilled water (1ml/kg)	3	-
II	5	3	-
III	50	3	-
IV	300	3	-
V	2000	3	-

Since there was no mortality of the animal in acute toxicity study, lethal dose of drug could not be calculated.

Table – 27: Sub – Acute Toxicity Analysis

Group	Dose (mg/kg)	Both rats	Days	No.of rats died
I	Control	3 Male + 3 Female	28	-
II	12.5	3 Male + 3 Female	28	-
III	25	3 Male + 3 Female	28	-
IV	50	3 Male + 3 Female	28	-

In case of Sub – Acute Toxicity Study, with the help of physiological parameters such as Haematological investigations and with the Histopathological studies the drug reaction within the animal can be assessed and are being tabulated respectively.

Lethal dose of the drug **"GANDHAGA MATHIRAI"**can be calculated with higher dose level of the drug which can be done in further studies.

8. DISCUSSION

The preclinical toxicity study of GANDHAGA MATHIRAI was conducted with the prime objective to find out whether the drug has possess any side effects or adverse reactions on long term administration.

Biochemical analysis of GANDHAGA MATHIRAI indicated the presence of calcium, sulphate, chloride, ferrous iron and Unsaturated compounds. Heavy toxic metals such as lead, mercury, arsenic, and zinc were absent.

Phytochemical analysis of GANDHAGA MATHIRAI shows the presence of carbohydrates, glycosides , tannins, alkaloids, fixed oil and fats .

FTIR study of GANDHAGA MATHIRAI shows the presene of functional groups such as Alkanes, Aldehydes, δ-lactone, Aromatic compounds, Acyl and phenyll, Aliphatic iodo and chloro compounds, Alkyl halides, Phosphines, Water.

Scanned Electron Microscope study of end product shows that the particles were stabilized and have irregular morphology. The particles were distributed in range 10µm and the size is below 5 µm

In acute toxicity study all the animals were active and did not showed any signs of toxicity. The motor activities were normal in all the 5 groups of animals. This acute toxicity study results reveals that GANDHAGA MATHIRAI was nontoxic upto a dose level of 2000mg/kg body weight of the animal.

Doses for sub-acute toxicity study were selected on the basis of acute toxicity study. The selected doses were 12.5mg/kg, 25mg/kg and 50mg/kg body weight of the animal.

In sub-acute toxicity study in low and middle dose (12.5mg/kg and 25mg/kg) no signs of toxicity were observed. No changes in the hematological parameters. There was no changes in food intake, water intake and body weight. No mortality occurred till the last day of the study.

Necropsy study of the major organs liver, kidney and heart showed no apparent change in colour. The texture of the organs maintained and the specimens were normal in low and middle dose (12.5mg/kg and 25mg/kg) on macroscopical examination when compared with that of the control group.

Histopathological examination revealed that normal architecture in comparison with control and treated animal.

Since, there was no mortality in both acute and sub-acute toxicity studies the lethal dose of the drug could not be calculated. The biostatistical analysis reveals that GANDHAGA MATHIRAI is safe up to a dose level of 2000mg /kg body weight of the animal.

9. SUMMARY

The ingredients of GANDHAGA MATHIRAI were purified and the drug was prepared according to the process mentioned in Anuboga vaidhya navaneetham (Part – 6, Pg.No. 89, Second Edition - 2002, Hakim P. Mohamed Abdulla Sahib) The drug was selected for evaluating the toxic effect and mortality when given in short and long duration. The aim of this study is to evaluate the safety of the drug GANDHAGA MATHIRAI by administering it to Wistar albino rats at various dose levels.

In review of literature, the ingredients of GANDHAGA MATHIRAI were discussed in depth with a special attention paid to their medicinal uses and toxicological aspects.

The ingredients of GANDHAGA MATHIRAI are gandhagam and changan leaf. The Gandhagam were purchased from Palani and Changan leaf collected from Pattukkottai.

The raw samples were taken for purification and test medicine was prepared, as per the method narrated in the literature.

Biochemical analysis of GANDHAGA MATHIRAI indicated the presence of calcium, sulphate, chloride, ferrous iron and Unsaturated compounds. Heavy metals such as lead, mercury, arsenic, and zinc were absent.

Phytochemical analysis of GANDHAGA MATHIRAI shows the presence of carbohydrates, glycosides , tannins, alkaloids, fixed oil and fats .

FTIR study of GANDHAGA MATHIRAI shows the presene of functional groups such as Alkanes, Aldehydes, δ-lactone, Aromatic compounds, Acyl and phenyll, Aliphatic iodo and chloro compounds, Alkyl halides, Phosphines, Water.

Scanned Electron Microscope study of end product shows that the particles were stabilized and have irregular morphology. The particles were distributed in range 10µm and the size is below 5 µm

In acute toxicity study all the animals were active and did not showed any signs of toxicity. The motor activities were normal in all the 5 groups of animals. This acute toxicity study results reveals that GANDHAGA MATHIRAI was nontoxic upto a dose level of 2000mg/kg body weight of the animal.

The Acute toxicity study was conducted to know single dose toxicity of GANDHAGA MATHIRAI on female Wistar Albino Rats. The study was conducted using 15 female Wistar Albino Rats. The female animals were selected for study of 6 weeks old with weight range of within ±20% of mean body weight at the time of

randomization. The groups were numbered as group I, II, III, IV and V and dose with control, 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg of GANDHAGA MATHIRAI . The drug was administered by oral route as single dose and observed for 14 days. Daily the animals were observed for clinical signs and mortality. Body weight of animals was recorded once in a week.

There were no physical and general behavioral changes observed in wistar albino rats of 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg to rats during 14 days.

Body weight of all animals did not reveal any significant change as compared to vehicle control group.

Food consumption of all group animals was normal

Mortality was not observed in all treated groups.

In Sub-acute toxicity animals were selected randomly grouped into four different groups containing minimum 6 animals (3 male + 3 female) per groups. The groups were numbered as group I, II, III and dose with control, 12.5mg/kg (low dose), 25mg/kg (Middle dose) and 50mg/kg (High dose) of GANDHAGA MATHIRAI . The GANDHAGA MATHIRAI was administered as single dose for 28 days and all animals were observed daily once. These observations were also performed on week ends.

The observations included clinical signs of toxicity, food intake, water intake, body weight. No signs of toxicity were observed. There was no significant changes in food intake, water intake and body weight. No mortality occurred till the last day of the study.

The blood samples are used to evaluate Hematological parameters (like RBC, WBC, HB,DC) and evaluate biochemical parameters (like SGOT, SGPT, ALP, UREA and CREATININE). No changes in haematological parameters and biochemical parameters in low and middle dose (12.5 mg/kg, 25 mg/kg) and mild changes in high dose (50mg/kg).

On completion of the 28days of drug administration, Wistar Albino Rats were sacrificed. In macroscopic examination the Heart, Kidneys and Liver organs were weighed. The organs were normal when compared with control group. Histopathological examination revealed normal architecture in comparison with control and treated animal in low and middle doses (12.5 mg/kg, 25 mg/kg) and mild changes in high dose (50mg/kg).

10. CONCLUSION

Gandhaga Mathirai was studied for its acute and sub-acute toxicity effect by using laboratory animals. In acute toxicity study, Gandhaga Mathirai did not produce any specific toxicity or mortality even at the dose of 2000mg/kg in rat. In sub-acute toxicity study, 12.5, 25 and 50mg/kg of Gandhaga Mathirai was used and it was administered once daily for 28 days through oral route. Gandhaga Mathirai did not alter the body weight, food intake and water intake during the study period. After 28 days the blood was collected and subjected to Hematological, liver, kidney & cardiac function test. Gandhaga Mathirai at 12.5 mg/kg, 25 mg/kg was found to be safe and did not alter any of the biochemical parameter, Haematology and Histopathology (Liver, kidney and Heart). Gandhaga Mathirai at 50mg/kg showed mild toxic effect by altering the biochemical parameters to liver, kidney and heart compared to vehicle treated groups.

From the study it was concluded that, low and middle dose (12.5 mg/kg, 25 mg/kg) of Gandhaga mathirai was found to be safe when administered orally in human for long administration.

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